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NAME: MOSKOWITZ, WILLIAM  
APPL NO:1 R29 HL38878-01A2  
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COUN DATE: 01/89

## Childhood Passive Smoking: Cohort Study of Cardiac Risk

**X R-29 FIRST AWARD**

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- M.D.

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Pediatrics

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A longitudinal comparison of the responses of preadolescent passive smoking and nonsmoking twins and their parents is proposed which will test the following hypotheses: (1) Genetic factors account for a significant proportion of the variation in the hematologic and cardiovascular determinants of systemic oxygen delivery. (2) Adaptive responses of the oxygen delivery system differ in the same individual before and after puberty. (3) Passive smoking in children is an incremental risk factor for the development of accelerated atherosclerotic/ischemic cardiovascular disease. (4) Passive smoking in children is a contributing factor in the development of reactive airway disease. A sample of 300 preadolescent twin pairs stratified by sex, zygosity and cigarette smoke exposure, will be recruited from an established population-based twin study. A resting and exercise noninvasive evaluation of hemodynamic (resting blood pressure, heart rate, echocardiographic evaluation of cardiac output and left ventricular mass, and exercise heart rate, blood pressure and oxygen consumption), hematologic (hematocrit and blood levels of 2,3-DPG, cotinine, thiocyanate and erythropoietin), hemorheologic (viscosity profile) and pulmonary (spirometry and flow/volume loops) components of the oxygen transport system will be performed. Serum lipoprotein levels will be measured. Three cohorts of 11-year-old twins will initially be evaluated in a cross-sectional study and then followed longitudinally for up to 3 years. The availability of repeated measures of oxygen delivery and its determinants in twins and their parents will permit both a unique analysis of genetic and environmental factors during the process of developmental change and measurements of the risks of accelerated atherosclerotic/ischemic heart disease and of the development of reactive airway disease.

William B. Moskowitz, M.D.	Principal Investigator	Dept. of Pediatrics Medical College of VA.
John K. Hewitt, Ph.D.	Co-Investigator	Dept. of Human Genetics Medical College of VA.
Richard M. Schieken, M.D.	Cardiology Consultant	Dept. of Pediatrics Medical College of VA.
Lindon J. Eaves, Ph.D.	Genetics Consultant	Dept. of Human Genetics Medical College of VA.
Walter E. Nance, M.D., Ph.D.	Genetics Consultant	Dept. of Human Genetics Medical College of VA.
Michael Mosteller, Ph.D.	Data Management Consultant	Dept. of Human Genetics Medical College of VA.

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U.S. GOVERNMENT PRINTING OFFICE: 1945 16-6330-1000

Number of Publications and Manuscripts accepted for publication (Not to exceed forty) 4  
See Remarks

**Appendix I. Publications**

**Appendix II. Written Agreement and Cost Structure for Assays**

**Appendix III. Structural Equation Modelling**

**Appendix IV. Letters of Reference for FIRST Award**

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# R: REDACTED MATERIAL

APPROVED BY RESEARCH PROGRAM DIRECTOR: William B. Moskowitz, M.D.					
FIRST 6-MONTH BUDGET PERIOD		FROM	TO		
DIRECT COSTS ONLY		4/1/89	3/31/90		
				AMOUNT	PERCENT
				AMOUNT	PERCENT
William B. Moskowitz, M.D.	Principal Investigator	1.0	50%	50%	REDACTED
John K. Hewitt, Ph.D.	Co-Investigator	1.0	10%	10%	REDACTED
To be Named	Lab Technician	1.0	100%	100%	REDACTED
Richard M. Schieken, M.D.	Consultant	1.0	2%	2%	0
Walter E. Nance, M.D.,PhD	Consultant	1.0	2%	2%	0
Linden J. Eaves, Ph.D.	Consultant	1.0	2%	2%	0
Michael Mosteller, Ph.D.	Consultant	1.0	2%	2%	0
 REDACTED R					
none					0
Waters MRM-1 Oxygen Consumption computer with adult and pediatric mask assemblies, oxygen cell, electrode gel, membrane kit and calibrator					12,000
Reagents and enzymes for 2,3-DPG assay					1,200
Reagents for colorimetric determination of thiocyanate					200
Disposable cuvettes, pipettes, capillary tubes, glassware					305
Oxygen sensors for oxygen consumption computer @ \$250 each					250
Disposable mouthpieces for spirometer					100
none					0
none					0
none					0
none					0
none					0
Computer time @ \$500./hour					500
Data Entry Forms					125
Postage for shipping of samples for assays					300
Cotinine Assay(\$16/, 200 assays) Erythropoietin Assay (\$55/, 100 assays)					8,700
TOTAL DIRECT COSTS FOR FIRST 6-MONTH BUDGET PERIOD Item 7a → \$ 75,027					
HS-13-Rev. 3/88		Page 4			
Fees quoted consecutively at the bottom throughout the application. Do not use suffixes such as Jr. Sr.					

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FEDERAL INVESTIGATOR PROGRAM DRAFTED: William B. Moskowitz, M.D.

3 BUDGET FOR ENTIRE PROPOSED PERIOD: 36 MONTHS  
DIRECT COSTS ONLY

ACCOUNT CATEGORY	BUDGET PER YR	ADDITIONAL YEARS OF SUPPORT REQUESTED			
1. PERSONNEL	\$51,347 (C-PI 14%)	\$50,713 (100-PI 5%)	\$55,537 (100-PI 10%)	\$57,758 (C-PI 10%)	\$63,016 (C-PI 15%)
2. EQUIPMENT	0	0	0	0	0
3. CONFERENCES	12,000	0	0	0	0
4. TRAVEL	2,055	2,055	2,055	2,055	2,055
5. DOMESTIC	0	0	0	0	0
6. FOREIGN	0	0	0	0	0
7. CONTRACTUAL	0	0	0	0	0
8. PURCHASES	0	0	0	0	0
9. LEASING	0	0	0	0	0
10. DIRECT COSTS	9,625	9,500	9,500	9,500	9,500
TOTAL DIRECT COSTS	\$75,027	\$62,273	\$67,255	\$69,692	\$75,755

348,276  
TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD Item 5a) → \$ 350,000

The proposed budget includes personnel, fringe benefits, and cost of living increases for the first five years. It also includes major equipment, design travel, alterations and renovations, patient care costs, and additional years of support. Requested annual merit increases in all categories over the first 12-month budget period will be approximately 5% per year. There will be no significant increase in personnel or other costs. The principal investigator will be the only laboratory supervisor. There will be no significant increases in any category over the current level of support.

Personnel

Salaries include 26% fringe benefits and 8% merit and cost of living increases from the first year through the fifth year in keeping with instructions from our grants management office. The indicated effort reflects a conservative estimate of the time that will be devoted to the project. The principal investigator and co-investigator will devote whatever time is necessary to insure the timely completion of the work described in this application.

William B. Moskowitz, M.D., Principal Investigator, is requesting \$20,000 a year (plus fringe benefits, without 8% yearly increase) due to the budgetary constraints of the FIRST Award. Starting July 1988, Dr. Moskowitz's ceiling salary as defined by the Medical College of Virginia will be \$98,936. Therefore the requested amount represents approximately 20% of his anticipated salary. Dr. Moskowitz will be responsible for the overall coordination and implementation of the project including analysis and publication of results. He will train and supervise the laboratory technician in the methods of pulmonary function testing, viscosity measurement and performance of the 2,3-DPG and thiocyanate assays.

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John K. Hewitt, Ph.D., Co-Investigator, will devote 10% effort toward the grant in the first year, 5% in the second year, 10% in the third and fourth years and 15% during the fifth and final year. Dr. Hewitt will be instrumental in the analysis of the data using developmental genetic analysis techniques. Dr. Hewitt along with Drs. Eaves and Nance are the major innovators of these methods of analysis which are used in this project.

Laboratory Technician A (TBA), will devote 100% effort to the project. In addition to the eventual performance of the pulmonary function tests, viscosity measurements and performance of the assays, the laboratory technician will participate in the acquisition and processing of all blood samples, the performance of exercise tests and the entry of data onto the data entry forms.

Richard M. Schieken, M.D., Consultant, will devote a 2% effort (without salary) toward the grant. Dr. Schieken is a nationally recognized expert in pediatric cardiovascular epidemiology and echocardiography and will provide expertise in these areas of the project.

Walter E. Nance, M.D., Ph.D., Consultant, will devote a 2% effort (without salary) toward the grant. Dr. Nance is a nationally recognized expert in twin genetics and will provide expertise in the genetic aspects of the project.

Lindon Eaves, Ph.D., Consultant, will devote a 2% effort (without salary) toward the grant. Dr. Eaves is an expert in epidemiological twin genetics and will provide guidance in the developmental and epidemiological aspects of the project.

Michael Mosteller, Ph.D., Consultant, will devote 2% effort (without salary) toward the grant. Dr. Mosteller is the Data Manager for the mother project and is involved in organizing the data for easy acquisition and maintaining the data sets. He will aid the Principal Investigator and Co-Investigator in the data management and analysis.

#### Equipment

The MRM-1 oxygen consumption computer provides an accurate continuous direct indication of oxygen uptake during exercise. The overall MRM-1 accuracy is conservatively stated as 5%. This compares favorably with the standard collection of expired air with Douglas bags with gas analysis.

#### Supplies

Reagents and enzymes for the colorimetric determination of both 2,3-DPG and thiocyanate will be purchased from Sigma Chemical Company. The budgeted amounts were calculated on an estimated 450 tests to be performed per year for each assay, as well as the monthly determination of standard and calibration curves. The glassware includes test tubes, pipets, and vacutainer for sample collection. Funds were also requested for disposable materials such as spirometry mouthpieces and oxygen sensors for the MRM-1.

#### Assay Costs

The fee-for-service arrangement exists for the performance of cotinine levels (\$16.00/assay) and erythropoietin levels (\$55/assay). Based on the observed 21% incidence of smokers in the pilot study, approximately 1 family per week would have cotinine levels measured. Over each year this represents approximately 200 assays. Erythropoietin levels will be obtained on passive smoking and nonpassive smoking monozygotic twins and their parents with an estimated 100 assays being performed each year.

#### Other Expenses

One hour of computer time will be required each year for data entry, storage, and manipulation as well as statistical analyses and graphic displays. Funds have been requested each year for the costs of shipping samples for cotinine and erythropoietin assays. Finally, a request for \$125 is made for preparation of data entry forms which will be available through the duration of the study.

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U.S.C.P.D. INVESTIGATOR PROGRAM DIRECTOR William B. Moskowitz, M.D.

GEOGRAPHICAL SKETCH

Indicate performance for the last personnel and consultant since 1. Beginning the American  
Investigator Program Protocol. "1" means "not yet" or "not yet".

NAME	POSITION	DATE OF DOB
William B. Moskowitz, M.D.	Assistant Professor Pediatric Cardiology	2/23/54
<hr/>		
EDUCATION	DEGREE	FIELD OF STUDY
Florida Technological University	B.S.	Microbiol/Biochem.
University of South Florida	M.D.	
Ped. Resident Children's Hosp. Philadelphia	1978-1981	
Fellow Ped. Cardiol. " " "	1981-1984	

EDUCATIONAL AND PROFESSIONAL EXPERIENCE Concluding with present position in chronological order. Previous employment, experience non-medical, include present membership in any Federal Government public advisory committee. List in chronological order the titles and corresponding publications during the past three years and/or reprints of five favorite publications pertaining to this occupation. DO NOT LIST PUBLICATIONS

Dr. Moskowitz was Board certified in Pediatrics in February, 1983 and Board certified in Pediatric Cardiology in October, 1985. He became a Fellow of the American Academy of Pediatrics in December, 1984 and Fellow of the American College of Cardiology in 1986.

Employment

1984-Present Assistant Professor Pediatric Cardiology, Medical College of Virginia

Honors

Alpha Omega Alpha, 1978; Outstanding Student in Pediatrics, 1978; Outstanding Student in Internal Medicine(Laennec Award), 1978; Outstanding Student in Radiology, 1978; Outstanding Teacher Award MCV Department of Pediatrics, 1986.

Publications

Harris MC, Moskowitz WB, Engle WD, Rosenberg H, Templeton J, Kumar S: Group B Streptococcal septicemia and delayed-onset diaphragmatic hernia. A new clinical association. Am J Dis Child 135:723-725, 1981.

Reich DR, Bernbaum J, Moskowitz WB: Passive delayed eruption of the primary dentition secondary to Dilantin administration. Oral Surg 52:599-601, 1981.

Tabak C, Moskowitz W, Wagner H, Weinberg P, Edmunds LH: Aortopulmonary window and aortic isthmus hypoplasia:operative management in newborns. J Thorac Cardiovasc Surg 86:273-279, 1983.

Moskowitz WB, Rashkind WJ: Hematologic and rheologic aspects of erythropheresis for polycythemia. Pediatric Research 18(4, Pt.2):127A, 1984.

Moskowitz WB, Gewitz MH, Heyman S, Ruddy RM, Scanlin TF: Cardiac involvement in cystic fibrosis: early noninvasive detection and vasodilator therapy. Pediatric Pharmacology 5:139-148, 1985.

Moskowitz WB, Hoyt RW: Echocardiography of elite rowers at rest and with exercise. Pediatric Research 20(4, Pt. 2):173A, 1986.

Moskowitz WB, Shula TJ, Schieken RM: Congenital aortic elongation: a rare cause of widened superior mediastinum. Am Heart J 112:1328-1330, 1986.

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Moskowitz WB, Clark BJ III: Cardiology and the primary care physician. Chapter in Primary Care Pediatrics, Schwartz W, Curry T, Charney E, Ludwig S (eds), Year Book Medical Publishers, 1987.

Moskowitz WB: Cardiac Emergencies in Pediatric Emergencies. Zanga J (ed). Churchill Livingston Inc.; New York, New York, 1987.

Moskowitz WB, Bodurtha JN, Mosteller M, Schieken RM: Is altered oxygen transport the mechanism for increased left ventricular thickness in passive smoking children? Pediatric Research 21(4, pt. 2):259A, 1987.

Schieken RM, Moskowitz WB, Mosteller M, Bodurtha JN: High blood pressure:A Hyperkinetic state? Circulation Vol 76 (4, pt.2), 492, 1987.

Moskowitz WB, Mosteller M, Bossano R, Schieken RM: Does passive smoking increase coronary heart disease risk in pubertal boys? Circulation Vol 76(4, pt.2), 359, 1987.

Moskowitz WB, Bossano R, Schieken RM: Altered diastolic function in children after successful coarctation repair. Pediatric Research Vol 22(4, pt. 2), 222A, 1988.

Schieken RM, Moskowitz WB, Bodurtha JN, Eaves L, Nance W: Aortic Stiffness: A new Doppler Echocardiographic Measure Predictive of Systolic Blood Pressure in Children. J American College Cardiology Vol 11; 1297-1300, 1988.

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## BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)	
John Keith Hewitt	Assistant Professor		
EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)			
INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
University of Birmingham, England	B.Sc.	1973	Psychology
University of Birmingham, England	M.Sc.	1974	Applied Genetics
University of London, England	Ph.D.	1978	Behavior Genetics
Open University, England	B.A.	1982	Mathematics

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

### Previous Employment and Experience:

1974-1977 Research Worker, Department of Psychology, Institute of Psychiatry, London.  
1977 Lecturer in Psychology, Department of Psychology, University of Birmingham.  
1985-1986 Visiting Associate Professor, Department of Biology, William Patterson College, New Jersey, USA.  
1986-pres Assistant Professor, Department of Human Genetics, Medical College of Virginia, Richmond, VA, USA.

### Honors:

- B.Sc. First class honors in Psychology

### Publications:

- Hewitt, J.K., Eysenck, H.J. and Eaves, L.J. The structure of social attitudes after twenty-five years: a replication. Psychological Reports. 1977, 40, 183-188.
- Hewitt, J.K., Fulker, D.W. and DeFries, J.C. Open-field behavior in mice: generality of results from a diallel analysis of replicated selection lines. Behavior Genetics. 1977, 7, 441-446.
- Hewitt, J.K. A note on the test for direction of dominance in the triple test cross in the presence of genotype x environment interaction. Heredity. 1980, 45, 293-295.
- Hewitt, J.K. and Fulker, D.W. Using the triple test cross to investigate the genetics of behavior in wild populations. I. Methodological considerations. Behavior Genetics. 1981, 11, 23-35.
- Hewitt, J.K., Fulker, D.W. and Broadhurst, P.L. Genetics of escape-avoidance conditioning in laboratory and wild populations of rats: a biometrical approach. Behavior Genetics. 1981, 11, 533-544. J
- Hewitt, J.K. and Broadhurst, P.L. Genetic architecture and the evolution of aggressive behavior. In E.C. Simmel, M.E. Hahn & J.K. Walters (Eds.). Aggressive behavior: Genetic and neural aspects. London, Lawrence Erlbaum. 1983.
- Hewitt, J.K. and Fulker, D.W. Using the triple test cross to investigate the genetics of behavior in wild populations. II. Escape- avoidance conditioning in Rattus norvegicus. Behavior Genetics. 1983, 13, 1-15.
- Hewitt, J.K., Fulker, D.W. and Hewitt, C.A. Genetic architecture of olfactory discriminative avoidance conditioning in Drosophila melanogaster. Journal of Comparative Psychology. 1983, 97, 52-58.
- Collins, M.F. and Hewitt, J.K. The genetic architecture of the male courtship sequence in Drosophila melanogaster. Heredity. 1984, 53, (2), 321-337
- Hewitt, J.K. Normal components of personality variation. Journal of Personality and Social Psychology. 1984, 47, 671-675.

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- Hewitt, J.K. and Fulker, D.W. Genetic differentiation of Roman selection lines did not disappear with inbreeding. Behavior Genetics, (1984) 14, 389-395.
- Hewitt, J.K., Carroll, D., Last, K., Turner, J.R. and Sims, J. A twin study of cardiac reactivity and parental hypertension (Abs.). Behavior Genetics. 1984, 14 (6), 604.
- Hewitt, J.K. and Fulker, D.W. Using the triple test cross to investigate the genetics of behavior in wild populations. III Activity and reactivity. Behavior Genetics. 1984, 14, 125-135.
- Hewitt, J.K. and Last, K. Twin studies of intelligence: Recent data, new issues. In C. Turner and M.B. Miles (Eds.), The biology of human intelligence. Nafferton: Nafferton Press, 1984.
- Carroll, D., Hewitt, J.K., Last, K.A., Turner, J.R. and Sims, J. A twin study of cardiac reactivity and its relationship to parental blood pressure. Physiology and Behavior, 1985, 34(1). 103-106.
- Collins, M.F. and Hewitt, J.K. Courtship between and within inbred lines of Drosophila melanogaster. Animal Behaviour, 1985, 33 (2). 682-683.
- Collins, M.F., Hewitt, J.K. and Gogarty, J.F. Validating time-sampled observations of courtship in Drosophila melanogaster for behavior genetic analysis. Behavior Genetics 1985, 15, 25-35
- Hallett, S., Quinn, D. and Hewitt, J.K. Defective interhemispheric integration and anomalous language representation in children at risk for schizophrenia. Journal of Nervous and Mental Diseases. 1986, 174, 418-427.
- Sims, J., Hewitt, J.K., Kelly, K.A., Carroll, D. and Turner, J.R. Familial and individual influences on blood pressure. Acta Geneticae Medicae et Gemmologiae, 1986, 35, 7-21.
- Turner, J.R., Carroll, D., Sims, J., Hewitt, J.K. and Kelly, K.A. Temporal and inter-task consistency of heart rate reactivity: a twin study. Physiology and Behavior, 1986, 38 (5), 641-644.
- Turner, J.R., Hewitt, J.K., Morgan, R.K., Sims, J., Carroll, D. and Kelly, K.A. Graded mental arithmetic as an active psychological challenge. International Journal of Psychophysiology, 1986, 3, 307-309.
- Hewitt, J.K. Heritability. Science Progress, Oxford, 1987, 71, 37-49.
- Carroll, D., Turner, J.R., Hewitt, J.K., Kelly, K.A. and Sims, J. Cardiac reactions to psychological challenge: implications for essential hypertension. In Taylor, E.W. (Ed.). Neurobiology of the cardiorespiratory system. Manchester University Press, in press.
- Claridge, G. and Hewitt, J.K. A biometrical study of schizotypy in a normal population. Personality and Individual Differences, in press
- Hahn, M.E., Hewitt, J.K., Adams, M. and Tully, T. Genetic influences on ultrasonic vocalization in the mouse. Behavior Genetics, 1987, in press.
- Hewitt, J.K., Hahn, M.E. and Karkowski, L. Genetic selection disrupts stability of mouse brain weight development. Developmental Brain Research, 1987, in press.
- Hewitt, J.K. and Heath, A.C. A note on computing the  $\chi^2$  noncentrality parameter for power analyses. Behavior Genetics, 1987, in press.
- Hewitt, J.K., Eaves, L.J. and Neale, M.C. Resolving causes of developmental continuity or "tracking". I. Longitudinal twin studies during growth. Behavior Genetics, accepted for publication.
- Eaves, L.J., Hewitt, J.K. and Heath, A.C. The quantitative study of human developmental change: A model and its limitations. 2nd International Conference on Quantitative Genetics, Sinauer Associates., 1988 (to be published)

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PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: William B. Moskowitz, MD

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Richard M. Schieken	Prof. & Chairman, Pediatric Cardiology, Dept. of Ped	

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (Circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
LaSalle College of Philadelphia (cum laude)	B.A.	1961	Biology
Univ. of Pennsylvania	M.D.	1965	Medicine
Ped. Intern, Children's Hosp. of Phil		1965-1966	Pediatrics
Ped. Assist, Chief Res. Child. Hosp. Phil		1966-1967	Pediatrics
Fellow, Ped. Card. Children's Hosp. of Phil		1968-1970	Pediatric Cardiology

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Employment

- 1966-1967 Asst. Instructor of Ped., Univ. of Pennsylvania School of Medicine  
1971-1973 Clinical Asst. Prof., Georgetown Univ. School of Medicine  
1973-1976 Asst. Prof. of Ped., Univ. of Iowa School of Medicine  
1976-1980 Assoc. Prof. of Ped., Univ. of Iowa School of Medicine  
1980-1982 Prof. of Ped., Univ. of Iowa School of Medicine  
1980-1982 Prof. of Prev. Medicine, Univ. of Iowa School of Medicine  
1982-Pres. Prof. & Chairman, Ped. Cardiology, Medical College of Virginia

Honors

Who's Who in Amer. Coll., 1961; Physician's Rec. Award, 1969; U.S. Army Commendation Medical, 1971; Nominated Teacher of the Year, 1976; Who's Who in the Midwest, 1977; Fellow of Seminar on Cardiovascular Epidemiology, 1979; Preventive Cardiology Academic Award, 1981-1982; Visiting Scholar, 1984-1985; School of Public Health, Univ. of N.C., 1981; NIH Study Section (EDC1) 1983-1987 (Chairman 1985-87); Executive Committee, National Cholesterol Education Committee, (NHLB1).

Publications (selected)

Mahoney, L.T., Schieken, R.M., Clarke, W.R., and Lauer, R.M.: The Development of Excessive Left Ventricular Mass in Children with Elevated Blood Pressure. 1987; In press.

Mahoney, L.T., Schieken, R.M., Clarke, W.R., and Lauer, R.M.: Left Ventricular Mass and Exercise Responses Predict Future Blood Pressure: The Muscatine Study. 1987; In press.

Weidman, W., Schieken, R.M. et. al: Diagnosis and Treatment of Primary Hyperlipidemia in Childhood. Circulation, Vol. 74, 1986; 1181A-1188A.

Meyer, J.M., Heath, A.C., Eaves, L.J., Mosteller, M., and Schieken, R.M.: The Predictive Power of Cattell's Personality Questionnaires: An Eighteen Month Prospective Study. In press.

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Moskowitz, W.B., Shula, T.J., Schieken, R.M. Congenital Aortic Elongation: A Rare Cause of Widened Superior Mediastinum. Am Heart J., 112:1328-1330, 1986.

Schieken, R.M., Lauer, R.M., Clarke, W.R. Hemodynamics in Childhood Hypertension. Clinical and Experimental Hypertension, Vol. A8, 703, 1986.

Schieken, R.M. Measurement of Left Ventricular Wall Mass in Pediatric Population. Hypertension, Supp II, 1987; 47-S2.

Bodurtha, J.N., Schieken, R.M., Segrest, J., Nance, W.E. HDL Cholesterol Subfractions in Adolescent Twins. Pediatrics, 1987; 79: 181-189.

Schieken, R., (1984) Effect of Blood Pressure on Left Ventricular Wall Mass in Filer, L.J., Jr. Lauer, R.M. (eds): Children's Blood Pressure, Report of the 88th Ross Conference on Pediatric Research. Columbus, Ohio: Ross Laboratories, 1985.

Schieken, R., (1983) Exercise Study of Blood Pressure: A Predictor of Future Hypertension? In NHLBI Workshop on Juvenile Hypertension, Ed. Loggie, J.M.H., Horan, M., Gruskin, A.B., et. al., Biomedical Information Corporation, NY, NY, 1984.

Schieken, R. (1983) Interrelation of Physical Activity and Nutrition on Blood Pressure in Diet and Exercise: Synergism Health Maintenance (White, P. and Mondeika, T. ed), 1983.

Schieken, R., Clarke, W., and Lauer, R.: The Cardiovascular Responses to Exercise in Children Across the Blood Pressure Distribution: The Muscatine Study. Hypertension, 5: 71-78, 1983.

Richardson, J.V., Schieken, R.M., Lauer, R.M., Stewart, P., Doty, D.B.: Repair of Large Ventricular Septal Defects in Infants and Small Children. Ann-Surg., March 195(3): pp. 318-22, 1982.

Schieken, R., Clarke, W., Prineas, R., Klein, V., and Lauer, R.: Electrocardiographic Measures of Left Ventricular Hypertrophy in Children Across the Distribution of Blood Pressure: THE Muscatine Study, Circ. 66: 428-432, 1982.

Schieken, R., Clarke, W., and Lauer, R.: Left Ventricular Hypertrophy in Children at the Upper Quintile of the Blood Pressure Distribution: The Muscatine Study, Hypertension, Vol. 3, No. 6, Nov/Dec 669-675, 1981.

Schieken, R., Clarke, W., and Lauer, R.: The Effect of Mitral Valvular Regurgitation on the Transthoracic Impedance Cardiogram. Br. Heart J. 45: 166-172, Feb. 1981.

Schieken, R., Mahoney, L., Clarke, W., and Lauer, R.: Measurement Criteria for Group Echocardiographic Studies. Am. J. Epidemiol. 110: 504, 1979.

Doty, D., Marvin, W., Schieken, R., and Lauer, R.: Hypoplastic Left Heart Syndrome Successful Palliation with a New Operation. J. Thorac. Cardiovas. Surg. 80: 148-152, July 1980.

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**BIOGRAPHICAL SKETCH**

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME  Walter E. Nance	TITLE  Professor and Chairman Dept. of Human Genetics	BIRTHDATE <u>Dec. 20, 1921</u>	
EDUCATION (begin with baccalaureate or other initial professional education and include postdoctoral training)			
INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
University of the South, Sewanee, TN Harvard Medical School, Boston, MA University of Wisconsin, Madison, WI	B.S. M.D. Ph.D.	1950-54 1954-58 1968	Mathematics Medicine Genetics

**RESEARCH AND/OR PROFESSIONAL EXPERIENCE:** Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

**Professional Experience:**

- 1963-69 Asst Prof. of Medicine & Head of the Div. of Medical Genetics, Vanderbilt University School of Medicine  
 1969-79 Prof. of Medical Genetics & of Medicine, Indiana School of Medicine  
 1975-Pres. Prof. & Chairman, Dept. of Human Genetics, Prof. of Medicine & Pediatrics, Medical College of Virginia

**Honors and Awards:**

Phi Beta Kappa, 1953; Graduated Optime Mores, 1954; Boylston Society Prize, 1958, Markle Scholar, 1964-65; American Society of Human Genetics: Board of Directors, 1966-68, 1985-87, Secretary, 1971-73; International Society for Twin Studies: Founding Member, 1977, Vice President, 1980-83, President, 1985-87; University Prize for Excellence in Teaching, Service and Research, 1985; Am. Soc. Clin. Invest., Genetic Soc. of America.

**Relevant Research:**

Dr. Nance is the Principal Investigator of NIH Grant AM 25786-07 titled "Genetic Epidemiology of Diabetes in Rubella Deafness."

**Bibliography:**

- Selected from a listing of 384 papers, abstracts, books and chapters.
- Nance, W.E.: Twins: An introduction to gemellology. Medicine, 38:403-414, 1959.  
 Nance, W.E.: Genetic control of hemoglobin synthesis. Science, 141:123-130, 1963.  
 Nance, W.E.: Genetic tests with a sex-linked marker: Glucose-6-phosphate dehydrogenase. Cold Spring Harbor Symp. on Quant. Biol., 29:415-425, 1964.  
 Nance, W.E. and Uchida, I.: Turner's syndrome, twinning, and an unusual variant of glucose- 6-phosphate dehydrogenase. Am. J. Hum. Genet., 16:380-392, 1964.  
 Nance, W.E.: Genetic studies of human serum and erythrocyte polymorphisms: Glucose-6-phosphate dehydrogenase, haptoglobin, hemoglobin, transferrin, lactin dehydrogenase a catalase, Ph.D. Thesis, University of Wisconsin, 1968.  
 Nance, W.E., Hara, S., Hasen, A., Elliott, J., Lewis, M. and Chown, B.: Genetic linkage studies in a Negro kindred with Norrie's disease. Am. J. Hum. Genet., 21:423-429, 1969.  
 Nance, W.E.: Anecephaly and spina bifida: A possible example of cytoplasmic inheritance in man. Nature, 224:373-375, 1969.  
 Nance, W.E., Reed, T. and Conneally, M.: Hb : Genetic linkage studies. Clin. Res., 18:395, 1970.  
 Kang, K.W., Smith, D.M., Johnston, C.C., Nakata, M. and Nance, W.E.: Is osteoporosis inherited? Clin. Res., 19:678, 1971.  
 McLeod, A.C., McConnell, F.E., Sweeney, A., Cooper, M.C. and Nance, W.E.: Clinical variation in Usher's syndrome. Arch. Otolaryng., 94:321-334, 1971.

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Bibliography (continued):

- Bader, P.I., Bingle, G.J., Yu, P.L., Clark, C.M. and Nance, W.E.: The inheritance of glucose tolerance as a quantitative variable. *Am. J. Hum. Genet.*, **25**:13a, 1973.
- Nance, W.E. and McConnell, F.E.: The status and prospects of research in hereditary deafness. *Adv. in Hum. Genet.*, **4**:173-250, 1973.
- Van Dyke, D.L., Palmer, C.G. and Nance, W.E.: Chromosome polymorphism and twin zygosity. *Am. J. Hum. Genet.*, **26**:88a, 1974.
- Nance, W.E. and Sweeney, A.: Genetic factors in deafness of early life. *Otol. Clinics of N. America*, **8**:19-48, 1975.
- Nance, W.E. and Corey, L.A.: Genetic models for the analysis of data from the families of identical twins. *Genetics*, **83**:811-826, 1976.
- Nance, W.E.: The role of twin studies in human quantitative genetics. *Prog. in Med. Genet. (New Ser.)* **3**:73-107, 1979.
- Heiberg, A., Magnus, P., Berg, K., and Nance, W.E.: Blood pressure in twins. *Proc. 3rd Int. Cong. of Twin Studies*, 1980.
- Røse, R.J., Boughman, J.A., Corey, L.A., Nance, W.E., Christian, J.C., and Kang, K.W.: Maternal effects on verbal intelligence date from the kinships of MZ twins. *Nature*, **283**:375-377, 1980.
- Phelan, M.C., Pellock, J.M., and Nance, W.E.: Discordant expression of fetal hydantoin syndrome in heteropaternal dizygotic twin. *N. Engl. J. Med.* **307**:99-102, 1982.
- Cantor, R.M., Nance, W.E., Eaves, L.J., Winter, P.M., and Blanchard, M.M.: Analysis of covariance structure of digital ridge counts in the offspring of monozygotic twins. *Genetics* **103**:495-512, 1983.
- Morton, C.C., Brown, J.A., Holmes, W.M., Nance, W.E., and Wolf, B.: Stain intensity of human nucleolus organizer region reflects incorporation of uridine into mature ribosomal RNA. *Exp. Cell Res.* **145**:405-413, 1983.
- Nance, W.E., Kramer, A., Corey, L.A., Winter, and Eaves, L.J.: A causal analysis of birth weight in the offspring of monozygotic twins. *Am. J. Hum. Genet.* **35**:1211-1223, 1983.
- Shaver, K.A., Boughman, J.A., Kenyon, N., Mohanakumar, T. and Nance, W.E.: HLA antigens in the congenital rubella syndrome. *Disease Markers* **2**:381-391, 1984.
- Magnus, P., Berg, K., Bjerkedal, T. and Nance, W.E.: Parental determinants of birth weight. *Clin. Genet.* **26**:397-405, 1984.
- Morton, C.C., Kirsch, I.R., Nance, W.E., Evans, G.A., Korman, A.J. and Strominger, J.L.: Orientation of loci within the human major histocompatibility complex by chromosomal *in situ* hybridization. *Proc. Nat. Acad. Sci.* **81**:2816-2820, 1984.
- Wolf, B., Heard, G.S., Jefferson, L.G., Proud, V.K., Nance, W.E. and Weissbecker, K.A.: Detection of biotinidase deficiency in a statewide neonatal screening program. *N. Engl. J. Med.* **313**:16-19, 1985.
- Magnus, P., Berg, K., Bjerkedal, T. and Nance, W.E.: The heritability of smoking behavior in pregnancy, and the birth weights of offspring of smoking-discordant twins. *Scand. J. Soc. Med.* **13**:29-34, 1985.
- Ostavik, K.H., Magnus, P., Berg, K. and Nance, W.E.: The genetic influence on the variation in plasma concentration of Factor VIII and Factor IX antigen. A twin study. *Am. J. Hum. Genet.* **37**:89-101, 1985.
- Shaver, K.A., Boughman, J.A., Nance, W.E.: Congenital rubella syndrome and diabetes: A review of epidemiologic, genetic, and immunologic factors. *Am. Ann. of Deaf* **130**:526-532, 1985.
- Bodurtha, J., Nance, W.E., Proud, V.K., and Townsend, I.: Updating McKusick: An educational exercise for medical students. *Am. J. Med. Genet.* **24**:505-511, 1986.
- Bodurtha, J., Nance, W.E.: The genetics of deafness. In *Otologic Medicine and Surgery*. Alberti, M.B., Ruben, R.J. (eds.), Churchill Livingston, New York, 1986 (in press).

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## BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo. Day Year)
Lindon J. Eaves	Distinguished Professor Department of Human Genetics	

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
University of Birmingham, England	BSc(Hon.CI)	1966	Genetics
Cuddesdon College, Oxford, England	GOE	1968	Theology
University of Birmingham, England	Ph.D.	1970	Behavioral Genetics
University of Oxford, England	MA(Oxon)	1979	Psychology
University of Birmingham, England	D.Sc.	1980	Genetics

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Positions:

- 1970-1979 MRC Research Fellow in Behavioral Genetics, Univ. of Birmingham, England.  
1978-1979 A.D. Williams Distinguished Visiting Prof., Med. College of Va., Richmond.  
1979 SRC Advanced Fellow in Genetics (Personal Fellowship awarded for distinction in Research) Univ. of Birmingham, England.  
1979-1981 University Lecturer (equiv. Associate Prof.), Oxford University, England.  
1981-pres Distinguished Prof. of Human Genetics, Medical College of Virginia, Richmond.

Honors:

- 1981 Holder of James Shields Award for Twin Research.

Publications:

-Dr. Eaves has participated in the preparation and publication of more than 70 papers, chapters, and reviews in the field of Behavioral and Statistical Genetics of which the following are relevant to the present proposal:

1. Eaves, L.J.: Computer simulation of sample size and experimental design in human psychogenetics. *Psychol. Bull.* 77:144-152, 1972.
2. Eaves, L.J., Jinks, J.L.: Insignificance of evidence for differences in heritability of IQ between races and social classes. *Nature* 240:84-88, 1972.
3. Eaves, L.J.: Assortative mating and intelligence: An analysis of pedigree data. *Heredity* 30:199-210, 1973.
4. Eaves, L.J., Eysenck, H.J.: Genetics and the development of social attitudes. *Nature* 249:288-289, 1974.
- 5. Eaves, L.J.: Testing models for variation in intelligence. *Heredity* 34:132-136, 1975.
- 6. Gale, J.S., Eaves, L.J.: The logic of animal conflict. *Nature* 254:463-464, 1975.
7. Eaves, L.J., Eysenck, H.J.: The nature of extraversion: A genetical analysis. *J. Person. and Soc. Psychol.* 32:102-112, 1975.
8. Eaves, L.J.: The effect of cultural transmission on continuous variation. *Heredity* 37:69-81, 1976.
9. Eaves, L.J.: A model for sibling effects in Man. *Heredity* 36:205-214, 1976.
10. Eaves, L.J.: Inferring the causes of human variation. *J. Roy. Statist. Soc.* 140:324-355, 1977.
- 11. Eaves, L.J., Last, K.A., Martin, N.G., Jinks, J.L.: A progressive approach to non-additivity and genotype-environmental covariance in the analysis of human differences. *Br. J. Math. Statist. Psychol.* 30:1-42, 1977.
12. Martin, N.G., Eaves, L.J.: The genetical analysis of covariance structure. *Heredity*, 38:79-95, 1977.
13. Martin, N.G., Eaves, L.J., Kearsey, M.J., Davies, P.: The power of the classical twin study. *Heredity*, 40:97-116, 1978.
14. Eaves, L.J., Last, K.A., Young, P.A., Martin, N.G.: Model-fitting approaches to the analysis of human behavior. *Heredity* 41:249-320, 1978.

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15. Young, P.A., Eaves, L.J. and Eysenck, H.J.: Intergenerational stability and change in the causes of variation in personality. *Personality and Individual Differences*, 1, 35-55, 1980.
16. Eaves, L.J.: The utility of twins. In V.E. Anderson (ed.), Genetic basis of the epilepsies. Raven, New York, 1982.
17. Eaves, L.J.: Errors of inference in the detection of major gene effects on psychological test scores. *American Journal of Human Genetics*, 35, 1179-1189, 1983.
18. Martin, N.G., Jardine, R. and Eaves, L.J.: Is there only one set of genes for different abilities? A re-analysis of the National Merit Scholarship Qualifying Test (NMSQT) data. *Behavior Genetics*, 15, 1211-1223, 1983.
19. Eaves, L.J.: The resolution of genotype x environment interaction in nuclear families. *Genetic Epidemiology*, 1, 215-228, 1984.
20. Heath, A.C., Berg, K., Eaves, L.J., Solass, M.H., Corey, L.A., Sundet, J., Magnus, P. and Nance, W.: Education policy and the heritability of educational attainment. *Nature*, 314, 734-736, 1985.
21. Heath, A.C., Berg, K., Eaves, L.J., Solass, M.H., Sundet, J., Nance, W.E., Corey, L.A. and Magnus, P.: No decline in assortative mating for educational level. *Behavior Genetics*, 15, 349-369, 1985.
22. Heath, A.C. and Eaves, L.J.: Resolving the effects of phenotype and social background on mate selection. *Behavior Genetics*, 15, 15-30, 1985.
23. Heath, A.C., Kendler, K.S., Eaves, L.J. and Markell, D.: The resolution of cultural and biological inheritance: Informativeness of different relationships. *Behavior Genetics*, 15, 439-465, 1985.
24. Corey, L.A., Eaves, L.J., Mellen, B.G. and Nance, W.E.: Testing for developmental changes in gene expression on resemblance for quantitative traits in kinships of twins. *Genetic Epidemiology*, 3, 73-83, 1986.
25. Kendler, K.S., Heath, A.C., Martin, N.G. and Eaves, L.J.: Symptoms of anxiety and depression in a volunteer twin population: The etiologic role of genetic and environmental factors. *Archives of General Psychiatry*, 43, 213-221, 1986.
26. Eaves, L.J., Kendler, K.S. and Schulz, S.C.: The familial sporadic classification: Its power for the resolution of genetic and environmental etiologic factors. *Journal of Psychiatric Research*, 20, 115-130, 1986.
27. Eaves, L.J., Long, J. and Heath, A.C.: A theory of developmental change in quantitative phenotypes applied to cognitive development. *Behavior Genetics*, 16, 143-162, 1986.
28. Martin, N.G., Eaves, L.J., Heath, A.C., Jardine, R., Feingold, L. and Eysenck, H.J.: The transmission of social attitudes. *Proceedings of the National Academy of Science*, 83, 4364-4368, 1986.
29. Kendler, K.S., Heath, A.C., Martin, N.G. and Eaves, L.J.: Symptoms of anxiety and symptoms of depression: Same genes, different environments? *Archives of General Psychiatry*, 44, 451-457, 1987.
30. Eaves, L.J., Martin, N.G. and Heath, A.C.: Religious affiliation in twins and their parents: Testing a model of cultural inheritance, submitted, 1987.
31. Eaves, L.J., Martin, N.G., Heath, A.C. and Kendler, K.S.: Testing genetic models for multiple symptoms: An application to the genetic analysis of liability to depression. *Behavior Genetics*, 17, Number 4, 1987.
32. Heath, A.C., Eaves, L.J., Nance, W.E. and Corey, L.A.: Social inequality and assortative mating: Cause or consequence? *Behavior Genetics*, in press.
33. Hewitt, J.K., Eaves, L.J. and Neale, M.C.: Resolving causes of developmental continuity or "tracking", submitted, 1987.

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PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: William B. Moskowitz, Jr.  
BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants listed on page 2. Begin with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME  <u>Michael Mosteller, Jr., Ph.D.</u>	POSITION TITLE  <u>Assistant Professor</u>	BIRTHDATE (Mo., Day, Yr.)	
<u>EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</u>			
INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
College of William and Mary Williamsburg, VA Virginia Commonwealth University Richmond, VA	BS  Ph.D.	1973  1982	Biology  Human Genetics

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience and honors. Include present membership on any Federal/Government public advisory committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1. Marsland, D.W., Nayfield, S.G., Johnson, R.E., Carter, W.H., Mosteller, M., Wood M., and Schieken, R.M.: Prevalence of hypertension in blacks versus whites across education and occupation in a primary care population. Proceedings of the 14th Annual Meeting of the North American Primary Care Research Group (Abstract), 632, 1986.
2. Meyer, J.M., Heath, A.C., Eaves, L.J., Mosteller, M., and Schieken, R.M.: The predictive power of Cattell's personality questionnaires: an eighteen month prospective study. Personality and Individual Differences (in press), 1987.
3. Mayo, F., Marsland, D., Wood, M., Mosteller, M., Miller, G.W., Johnson, R.E., Munson, P.J.: Denominator definition by the utilization factor method. Family Practice: an International Journal, 3(3):184-191, 1986.
4. Moskowitz, W.B., Bodurtha, J.N., Mosteller, M., and Schieken, R.M.: Is altered oxygen transport the mechanism for increased left ventricular thickness in passive smoking children? Pediatric Research, 21(4, pt. 2), 1987.
5. Mosteller, M., Mamunes, P., and Brown, J.A.: Wolf-Hirschorn Syndrome: report of two cases including one with a sporadic translocation. Clinical Research (Abstract), 26(1): 75A, 1978.
6. Mosteller M., Townsend, J.I., Corey, L.A., and Nance, W.E.: Twinning Rates in Virginia: Secular Trends and the Effects of Maternal Age and Parity in Twin Research 3: Twin Biology and Multiple Pregnancy, Luigi Gedda, Paolo Parisi, and Walter E. Nance, Editors. Alan R. Liss, Inc., New York, 1981.
7. Nance, W.E., Corey, L.A., and Mosteller, M.: A new method for detecting pleiotrophisms using data from twins. American Journal of Human Genetics (Abstract), 39:139A, 1980.
8. Nix, M.L., Balcksher, B., Pomfrey, B.J., Guidt, P., Mosteller, M., and Barnes, R.W.: Noninvasive technology transfer for epidemiologic study of carotid artery disease. Bruit., 10:161-164, 1986.
9. Schieken, R.M., Eaves, L., Nance, W.E., Mosteller, M., and Bodurtha, J.: The genetics of aortic tone in children. Circulation (Abstract), 74(4, pt. 2):1-36, 1986.

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**OTHER SUPPORT**

Use continuation pages, if necessary.

**INSTRUCTIONS CAREFULLY**: Incomplete, inaccurate, or ambiguous information about OTHERS SUPPORT could lead to delays in the review process. There are changes subsequent to submission, notify the scientific secretariat of the review group.

1. If there is no separate budget for the other support, list current: (a) amount and (b) applications and proposals submitted during the last year and (c) amount of time spent preparing the application. To be an Federal Non-Federal organization, foundation, corporation, contract, and fellowship support at the local, state, organization and elsewhere. If particular, cite the date, name of the principal investigator, program director and provide the data for each project; the funding the sub-project, if any.

2. At item level: a) the source of support; identifying number and title; (b) percentage of appointment on the project; (c) dates of entry; (d) percent of annual direct cost; (e) brief description of the project; (f) whether the item overlaps, duplicates, or is being replaced by the present application; (g) create and justify the nature and extent of any scientific and/or budgetary overlaps or boundaries; (h) any modifications that will be made should the present application be funded.

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR:

1. **CURRENT ACTIVE SUPPORT**:

Title:	Longitudinal Twin Study-Cohort Study of Blood Pressure
Program Director:	Richard M. Schieken, M.D.
Effort:	10%
Annual Direct Cost:	\$2,677,877
Grant Number:	RO1-MH 31010
Period:	8/1/88-7/31/93

John K. Hewitt, Ph.D. (15% OHS)

Title:	Resolving Power of Kindship Analysis for Genetic Research
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Program Director:	Lindon Eaves
Grant Number:	NIH GM 30250
Effort:	10%
Annual Direct Cost:	\$75,113
Period:	2/86-1/91

Title:	Genetic Models of Development and Aging
--------	---

Program Director:	Lindon Eaves
Grant Number:	NIH MH 40828
Effort:	17%
Annual Direct Cost:	\$51,819
Period:	7/84-6/88

Title:	Biological and Cultural Determinants of Alcohol Consumption
--------	---

Program Director:	Andrew Heath
Grant Number:	NIH AA-06781
Effort:	20%
Annual Direct Cost:	\$95,168
Period:	8/85-7/89

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## John K. Hewitt, Ph.D. (cont.)

Title: Epidemiology of Anxiety and Depression  
 Program Director: Kenneth Kendler  
 Grant Number: NIMH MH-40828  
 Effort: 20%  
 Annual Direct Cost: \$383,315  
 Period: 2/86- 1/90

## Applications Pending

Title:  
 Program Director:  
 Effort:  
 Annual Direct Cost:  
 Period:

Title:  
 Program Director:  
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Title:  
 Program Director:  
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 Annual Direct Cost:  
 Period:

Richard M. Schieken, M.D.

Title:  
 Program Director:  
 Effort:  
 Annual Direct Cost:  
 Period:

Title:  
 Program Director:  
 Grant Number:  
 Effort:  
 Annual Direct Cost:  
 Period:

Title: Longitudinal Twin Study: Cohort Study of Blood Pressure  
 Program Director: Richard M. Schieken, M.D.  
 Effort: 20%  
 Grant Number: NHLBI R01-HL 31010  
 Annual Direct Cost: \$885,748  
 Period: 8/1/83- 7/31/88 (renewal pending)

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- Richard M. Schieken, M.D. (cont.)

Applications pending

Title:  
 Program Director:  
 Effort:  
 Grant Number:  
 Annual Direct Cost:  
 Period:

Walter E. Nance, M.D., Ph.D.

Title: MCV Predoctoral Training Grant in Human Genetics  
 Program Director: Walter E. Nance  
 Grant Number: NIH 6M-07492  
 Effort: 10%  
 Annual Direct Cost: \$45,072  
 Period: 7/77- 6/87

Title:  
 Program Director:  
 Effort:  
 Annual Direct Cost:  
 Period:

Title: Clinical Research Center for Periodontal Disease  
 Program Director: Harvey Schenkein, D.D.S.  
 Grant Number: NIH DE-05139  
 Effort: 10%  
 Annual Direct Cost: \$80,323  
 Period: 8/78- 7/88

Title:  
 Program Director:  
 Grant Number:  
 Effort:  
 Annual Direct Cost:  
 Period:

Genetic Testing and Counseling Program  
 Walter E. Nance  
 HSA/PDI5-047  
 Not specified  
 \$155,701  
 Continuous

Title:  
 Program Director:  
 Grant Number:  
 Effort:  
 Annual Direct Cost:  
 Period:

Genetic Epidemiology of Diabetes Rubella Deafness  
 Walter E. Nance  
 NIH AM257806-02  
 10%  
 \$120,545  
 12/79- 8/88

Title:  
 Program Director:  
 Grant Number:  
 Effort:  
 Annual Direct Cost:  
 Period:

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**PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: William B. Moskowitz, MD**

**Walter E. Nance, M.D., Ph.D. (cont.)**

Title: Longitudinal Twin Study:Cohort Study of Blood Pressure  
Program Director: Richard M. Schieken  
Effort: 10%  
Grant Number: NHLBI R01-HL 31010  
Annual Direct Cost: \$885,748  
Period: 8/1/83- 7/31/88

**Applications pending**

Title: Longitudinal Twin Study: Cohort Study of Blood Pressure  
Program Director: Richard Schieken  
Effort: 5%  
Grant Number: NHLBI R01-HL 31010 (*Renewal*)  
Annual Direct Cost: \$2,677,877  
Period: 8/1/88- 7/31/93

**Lindon Eaves, Ph.D.**

Title:  
Program Director:  
Effort:  
Annual Direct Cost:  
Period:

Title: Resolving Power of Kinship Analysis for Genetic Research  
Program Director: Lindon Eaves  
Grant Number: NIH GM 30250  
Effort: 20%  
Annual Direct Cost: \$75,113  
Period: 2/86- 1/91

Title: Genetic Models of Development and Aging  
Program Director: Lindon Eaves  
Grant Number: NIH MH 40828  
Effort: 14%  
Annual Direct Cost: \$51,819  
Period: 7/84- 6/88

Title: Periodontal Disease: Microbiological Studies  
Program Director: Richard R. Rainey D.D.S.  
Grant Number: NIH DE-05054  
Effort: 6%  
Annual Direct Cost: \$332,446  
Period: 3/85- 2/90

Title: Biological and Cultural Determinants of Alcohol Consumption  
Program Director: Andrew Heath  
Grant Number: NIH AA-06781  
Effort: 5%  
Annual Direct Cost: \$95,168  
Period: 8/85- 7/89

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Lindon Eaves, Ph.D. (cont.)

Title: Epidemiology of Anxiety and Depression  
 Program Director: Kenneth Kendler  
 Grant Number: NIHMH MH-40828  
 Effort: 5%  
 Annual Direct Cost: \$382,315  
 Period: 2/86- 1/90

Title:  
 Program Director:  
 Grant Number:  
 Effort:  
 Annual Direct Cost:  
 Period:

Title: Longitudinal Twin Study: Cohort Study of Blood Pressure  
 Program Director: Richard M. Schieken  
 Grant Number: NHLBI RO1-HL 31010  
 Effort: 10%  
 Annual Direct Cost: \$885,748  
 Period: 8/1/83- 7/31/88

Applications pending

Title: Longitudinal Twin Study: Cohort Study of Blood Pressure  
 Program Director: Richard M. Schieken  
 Grant Number: NHLBI RO1-HL 31010 (*Renewal*)  
 Effort: 10%  
 Annual Direct Cost: \$2,677,877  
 Period: 8/1/88- 7/31/93

Title:  
 Program Director:  
 Effort:  
 Annual Direct Cost:  
 Period:

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 Annual Direct Cost:  
 Period:

Michael Mosteller, Ph.D. ✓

Title: Cardiovascular Risk Reduction  
 Program Director: Richard M. Schieken  
 Grant Number: NHLBI R18HL28922  
 Effort: 100%  
 Annual Direct Cost: \$4,440,280  
 Period: 3/31/82- 4/30/87 (ext. 7/31/88)

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Michael Mosteller, Ph.D. (cont)

Applications pending

Title: Longitudinal Twin Study: Cohort Study of Blood Pressure  
Program Director: Richard M. Schieken  
Grant Number: NHLBI R01-HL 31010  
Effort: 98%  
Annual Direct Cost: \$2,677,877  
Period: 8/1/88- 7/31/93

NIH Grant Application Form - Part II - Budget & Financial Management

2025793672

**RESOURCES AND ENVIRONMENT**

**FACILITIES:** Mark the facilities to be used at the applicant organization and briefly indicate their capacities, pertinent capabilities, relative proximity and external availability to the project. Use "Other" to describe the facilities at any other performance sites listed in item 9, page 1, and at sites for field studies. Using transportation codes if necessary, include an explanation of any consortium/contractual arrangements with other organizations.

Human Physiology Laboratory (585 sq ft) exclusively used by Pediatric Cardiology (anthropometrics, exercise tests, spirometry, venipuncture). Immunogenetic Laboratory-fully equipped immunogenetic laboratory  
 **Office:** available for zygosity determination  
Hemorheology Laboratory-(200 sq ft) exclusively used for Pediatric Cardiology (assays, viscosity profiles, hematocrit determinations)

**Clinical:**

**Animal:**

**Computer:** The following university computer resources are utilized to process research data:  
-An IBM-3081D, 24MB of memory, 3350 & 3380 disk drives & tape drives  
-A VAX 11/780 with 10 MB of memory, 9751 & 9758 disk drives & tape drives  
 **Office:** -SAS system software including base SAS and SAS/GRAF  
-TSO (Time-Sharing option) which allows on line access to mainframe datasets  
Office space (280 sq ft) for secretarial use and data reduction.

**Other:** \_\_\_\_\_

**MAJOR EQUIPMENT:** List the most important equipment items already available for this project, noting the location and pertinent capabilities of each. All of the following equipment are used exclusively for Pediatric Cardiology research:  
SKII ultrasonoscope A- m-mode echocardiograms, Summagraphics Bit Pad One-coupled to IBM-PC to measure and enter echo data in an unbiased fashion. MultiSPIRO-PC-computerized spirometry system used to generate flow/volume loops. Cone plate digital viscometer-measures blood viscosity at varying shear rates. Spectrophotometer- used during quantitative determinations of 2,3-DPG and thiocyanate, hematocrit centrifuge, (3)-test tube centrifuges for sample processing and standard and -70 freezers for samples

**ADDITIONAL INFORMATION:** Provide any other information describing the environment for the project. Identify support services such as consultants, secretaries, machine shop and electronics shop and the extent to which they will be available to the project.  
Secretarial support and the cost of telecommunications and document duplication will be provided by the Division of Pediatric Cardiology. The pilot study data were generated with the help of limited additional funds provided by the Division of Pediatric Cardiology. The four Consultants listed on page 2 and described on page 6 will provide guidance and assistance to the principal and co-investigator in their areas of expertise and will be available throughout the period of the grant.

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**SECTION 2. Introduction:**

This R-29 (FIRST) grant application represents a revised application, number 1 R29 HL 38878-01A1.

This Introduction and Section D exceeds by a small margin the page limitations set in the instructions. We exceeded the limitations to present a complete response to the criticisms in the Summary Statement. We apologize to the reviewers for this added burden.

Substantial revisions have been made in the grant application and these changes are identified within the text of the application by bold typography format. Data that were not available in the prior version have been included in this application, in bold format. Responses to criticisms in the previous Summary Statement are presented.

**1) What evidence exists that the follow-up evaluation of the twins will provide data of importance?** Cigarette smoking is a known powerful risk factor for the development of atherosclerotic coronary heart disease. Twenty-one percent of our twin population has been chronically passively exposed to this risk factor. Longitudinal twin data collected prior to and throughout puberty and into adolescence, together with repeated measurements on the parents will give us a unique data set for analysis.

In its simplest form, a classical twin study involves a comparison of the similarities of genetically identical monozygotic (MZ) twins and dizygotic (DZ) pairs who share, on the average, only half their genes. The extent to which the former exceeds the latter provides a measure of the importance of genetic differences as a cause for variation in the trait in question. Our initial studies, performed when all twins are 11 years of age, will allow 2 types of studies: 1) epidemiologic association and 2) univariate genetic analysis. The longitudinal studies (ages 12.5 and 14 years) will allow us to search for causal mechanisms and to determine whether different genes are responsible for these mechanisms in later puberty or if additive genetic or additive environmental effects cause changes in the response to passive smoking. Extension of the research design to include longitudinal observations in unlike-sexed DZ pairs permits assessment of the consistency of genetic effects across the sexes. In addition, a proportion of twins will become active smokers during puberty, thereby increasing their specific environmental determinants. Because they exploit the closest possible genetic relationship, twin studies are more efficient than any alternative research design and are, thus, ideally suited for the longitudinal genetic analysis of complex physiologic responses, which exist in the oxygen transport system.

**2) There is no supportive information for hypothesis 2 under "significance," nor is this issue addressed in other portions of the application.** Adaptive responses within the oxygen delivery system differ between the sexes and in the same individual during developmental growth through adolescence. Supportive information has been added to Section 2.B. (see ref. 6,7) and is identified within the text by bold format. Preliminary data in support of this hypothesis are now available and have been included in this application.

**3) A second area of concern is the regression model. It is possible that this model loses the replication aspect of twins. This should be a mixed (fixed/random) effects model where interclass correlation as a function of independent variables is of interest, as well as regression coefficients themselves. It is not clear exactly what is meant here.**

One interpretation is that the replicate observations provided by members of a twin pair are helpful in assessing the ability of a regression model to predict criterion variables such as LV mass or diastolic blood pressure from independent variables such as smoking status (fixed effects) and covariates such as body weight (random effects). The adequacy of the predictor variables (independent variables and covariates)

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taken together is indexed by the squared multiple correlation ( $R^2$ ) between the predictor variables and the criterion; the independent contribution of a particular variable may be judged by how much it increments  $R^2$ . This analysis focuses on the interclass correlations as a function of the independent variables of interest and may be implemented using, for example, a stepwise multiple regression procedure (e.g. PROC STEPWISE in SAS) which provides the relevant statistics and appropriate tests of significance.

However, the success of the prediction equation may be overestimated in any particular sample because we "capitalize on chance" associations in the sample data. The prediction equation uses regression weights which maximize the correlation between predicted and observed values. Thus to avoid an overoptimistic interpretation of the success of the prediction equation, a recommended safeguard is to test the prediction equation on another sample not used in deriving the regression weights. Short of recruiting a new sample, and doubling the size and cost of the study, we may randomly select one member from each twin pair to serve as our "estimation" sample to derive the prediction equation and use the other member of each pair as our "test" sample to evaluate the  $R^2$ . Although these two samples are not independent, in as much as the  $R^2$  was inflated in the "estimation" sample purely by the association of random individual errors of measurement, so the  $R^2$  will be corrected downwards in the "test" sample. This makes use of the replication aspect of twins to provide a check on over interpretation of the data.

As an illustration of this we looked at the prediction of resting systolic blood pressure (SBP) from age, resting heart rate and weight in one twin from each complete pair in our pilot sample. The  $R^2$  was 0.23 and was highly significant ( $p < .0001$ ). Using the derived prediction equation to predict the SBPs of the second twin from each pair resulted in an  $R^2$  of 0.21 which was, of course, similarly significant. We conclude that these predictor variables account for over 20% of the variation in SBP and that this level of prediction is not a consequence of associated errors of measurement in the sample used to derive the prediction equation.

4) The importance and significance of the research on genetic and environmental influences on oxygen delivery are not well described.

These elements are clearly outlined and detailed extensively in the Significance and Preliminary Studies sections of this application.

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**Section 2. RESEARCH PLAN**  
**A. Specific Aims**

**Hypotheses**

A longitudinal comparison of the cardiovascular, hematologic, hemorheologic, pulmonary and lipoprotein responses of preadolescent passive smoking and nonsmoking twins and their parents is proposed which will allow us to test the following hypotheses:

1. Genetic factors account for a significant proportion of the variation in the hematologic and cardiovascular determinants of systemic oxygen delivery.
2. Adaptive responses within the oxygen delivery system differ between the sexes and in the same individual before and during puberty.
3. Passive smoking in children is an incremental risk factor for the development of accelerated atherosclerotic/ischemic cardiovascular disease.
4. Passive smoking in children is a contributing factor in the development of reactive airway disease.

**Specific Goals**

1. To compare the resting heart rate, systolic and diastolic blood pressures of preadolescent passive smoking twins with age, sex and anthropometrically matched nonsmoking twins.
2. To measure and compare oxygen delivery components (hematocrit, 2,3 DPG concentration, oxygen consumption and cardiac output) in preadolescent monozygotic and dizygotic sex matched passive smoking and nonpassive smoking twins and their parents.
3. To measure and compare specific responses of the oxygen delivery components in twins before and during puberty.
4. To correlate measured differences in oxygen delivery components with serum thiocyanate and cotinine levels in all family members.
5. To measure and compare whole blood viscosity in passive smoking and nonpassive smoking preadolescent twins and their parents.
6. To determine the effect of whole blood viscosity on left ventricular mass in passive smoking and nonpassive smoking individuals after adjusting for body size.
7. To compare the exercise responses of preadolescent passive smokers to age, sex and anthropometrically matched nonpassive smokers.
8. To perform and compare pulmonary flow/volume loops before and following exercise in passive and nonpassive smoking twins and their parents.
9. To correlate the extent and severity of the cardiovascular, hematologic, hemorheologic and pulmonary alterations with the duration and extent of passive smoking exposure.
10. To test for shared environmental effects (passive smoking) upon serum lipid and lipoprotein levels in twin pairs.
11. To measure and compare serum erythropoietin levels in active, passive and nonsmoking individuals.
12. To correlate erythropoietin levels with cigarette smoke exposure (cotinine and thiocyanate levels) and measured components of the oxygen delivery system.

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**B. Significance**

The adverse health effects of actively inhaled cigarette smoke include impaired pulmonary function, increased coronary and cerebrovascular disease, chronic pulmonary disease and cancer. (1-3) Infants and young children of smoking parents are at increased risk for lower respiratory tract infections and small airways disease than are children of nonsmoking parents. (4,5) What is less clear is how the oxygen transport system of the growing child is affected by the long-term exposure to and passive inhalation of cigarette smoke and if this exposure represents a risk for the subsequent development of atherosclerotic heart disease.

Systemic oxygen delivery is comprised of and varies with a number of factors: oxygen tension of expired air, pulmonary function, hemoglobin concentration, affinity of hemoglobin for oxygen, speed of dissociation of oxygen from hemoglobin and cardiac output. The rate of erythropoiesis is governed by the rate of systemic oxygen transport- when oxygen transport decreases, erythropoiesis increases. In situations of impaired erythropoiesis, compensatory mechanisms (acceleration of heart rate and respiratory rate, increases in cardiac output and a shift to the right in oxygen saturation curve by increases in 2,3 diphosphoglycerate) are mobilized until the steady state is restored.

Developmental changes occur within the oxygen delivery system in the growing child. Cardiac output and oxygen consumption per body size decrease with growth. Among prepubertal children, hemoglobin and hematocrit values increase with age, with no significant sex differences. (6) During puberty and late adolescence, the values decrease in females and increase in males. Androgenic hormones are involved in these differences though the mechanism(s) has not been fully elucidated. Growing males demonstrate an accelerated postpubertal expansion of red cell mass. Females, due to a greater proportion of fat at a given body size before, especially during, and after puberty, demonstrate a smaller blood volume per kilogram with growth.(7) The mild environmental hypoxia at Denver which provided a stimulus in the adult to erythropoiesis did not seem to be operative in prepubertal boys or girls. Therefore, developmental changes in the oxygen delivery system occur within individuals and between sexes and adaptive responses to environmental stresses (high altitude hypoxia or chronic tobacco smoke exposure) may differ in children and adults.

Tobacco smoke exposure is cited as the major source of elevated carboxyhemoglobin in man and chronically elevated carboxyhemoglobin (COHb) levels play a role in the pathogenesis of atherosclerosis. Carbon monoxide is constantly produced endogenously in normal individuals as a by-product of heme protein catabolism and leads to COHb levels of only approximately 0.5%. (8) Smokers have much higher levels of COHb than do nonsmokers, and in a smoke contaminated environment achieve even greater increases in COHb levels.(9) Carboxyhemoglobin has an in-vivo half life of at least 3-4 hours which extends the period of effect of exposure to CO and provides for a build-up of COHb from fresh exposures.

Inhaled CO can cause tissue hypoxia.(10) Carbon monoxide combines readily with hemoglobin because its chemical affinity for hemoglobin is over 200 times greater than that of oxygen. The ability of CO to cause tissue hypoxia is related to two effects of COHb; 1) a reduction in the total amount of oxygen carried by red cells and 2) a shift to the left of the oxyhemoglobin dissociation curve which contains both oxy- and carboxyhemoglobin. Astrup observed that smokers could have a higher oxygen affinity of hemoglobin than that corresponding to the Haldane effect of present COHb.(11) A fall in red cell 2,3-DPG leads to a displacement of the oxyhemoglobin curve to the left.

In contrast to decreased 2,3-DPG levels in response to acute, marked elevations in COHb, most studies of chronic active smokers indicate a definite and significant increase in 2,3-DPG levels.(12,13) The 2,3-DPG

has its highest affinity for reduced hemoglobin and equal affinity for oxy- and carboxyhemoglobin. A hypoxia-driven mechanism to trigger 2,3-DPG synthesis may be responsible for the increase in 2,3-DPG level in smokers. Despite the lower levels of CO in passive smokers as compared to active smokers, oxygen transport impairment may exist which may lead to a compensatory increase in 2,3-DPG.

Reduced oxygen carrying capacity from CO exposure will limit the amount of oxygen available to active muscles during exercise. Individuals exposed to CO during maximal exercise have a significant reduction in maximal oxygen consumption in spite of higher heart rates and relative hyperventilation. (14,15) The reduction in maximal oxygen consumption paralleled the reduction of the blood oxygen transport capabilities.

The effect of passive smoking on maximal work capacity and exercise-induced angina has been studied. (16) Angina patients exposed to cigarette smoke increased their resting heart rate, systolic and diastolic blood pressure and venous COHb and decreased the duration of exercise until angina. The effect of smoking a single cigarette on regional myocardial perfusion was studied in chronic smokers with stable angina using positron emission tomography and rubidium-82 scintigraphy. (17) Smoking a single cigarette produced a profound momentary disturbance of regional myocardial blood flow similar to ischemic disturbances induced by physical exercise though the events during smoking were clinically silent. Such repeated insults possibly represent an important mechanism linking smoking with coronary events. Chronic passive smoking may have similar insidious effects on myocardial oxygen supply.

Increased COHb concentrations in active smokers have been associated with an increased erythrocyte mass. (18) The known causes of elevation of hematocrit values in active smokers have been described and chronic exposure to even low levels of CO can result in tissue hypoxia and lead to an increase in the red cell mass. (19) Erythropoiesis appears to be a major adaptive response in adult smokers to chronically reduced oxygen-carrying capacity. Whether this response occurs in childhood passive smokers before, during, or after puberty is unknown. Longitudinal measurement of hematocrit and serum erythropoietin levels, as well as other oxygen transport factors in pre-pubertal passive smoking and nonsmoking children will elucidate the active mechanisms of adaptation in childhood.

Tissue hypoxia is sensed by the kidney, which responds with the production and/or release of erythropoietin, a glycoprotein that functions as the major regulator of red cell production. (20) A radioimmunoassay for erythropoietin is available which achieves a sensitivity of 1 mU, equal to or greater than that obtained with most bioassays. (21) Bleeding of a normal individual increases the serum erythropoietin level and transfusion decreases it. When sickle cell disease patients receiving oxygen therapy had oxygen discontinued, levels rose measurably within three hours and reached maximal concentrations at 24-48 hours. (22) If a state of relative tissue hypoxia exists in active and passive smokers, the serum erythropoietin level may be elevated when compared to nonsmoking individuals and be proportional to the degree of tissue hypoxia.

In addition to an elevated hematocrit and red cell mass, active smokers demonstrate elevated fibrinogen levels and blood viscosity and increased aggregation of red cells and plasma viscosity when compared to age, sex, and weight matched nonsmokers. (23) Relative tissue hypoxia from COHb exposure could possibly be worsened by a concomitant elevation in blood viscosity. High rates of cardiovascular morbidity have been reported in patients with hyperviscosity due to hypertension. (24) Significant left ventricular (LV) hypertrophy as measured by echocardiography developed in hypertensive patients with elevated whole blood viscosity, whereas it did not develop in those with normal viscosity. (25) The mechanisms of this

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association may include either a primary pathogenic role of increased viscosity as a stimulus to hypertrophy, or a close parallelism between the development of elevated blood viscosity and increased LV mass in response to a common stimulus, such as CO. The severity of the myocardial hypertrophy in rats chronically exposed to CO is inversely proportion to the age of the animals and this excess myocardial mass persists into adulthood, long after termination of CO inhalation. (26) It is quite possible that similar mechanisms are active in passive smoking children, increasing their risk for cardiovascular disease in later life.

Although the aforementioned changes in oxygen delivery are potential causes of ischemic cardiovascular disease, there is good evidence that cigarette smoking alters the total serum cholesterol(C) and lipoprotein composition in ways that would increase the risk of atherosclerosis. The incidence of coronary artery disease is directly related to levels of LDL-C and inversely related to levels of HDL-C, especially HDL2-C. (27,28) Women generally have higher HDL-C levels in comparison to men, black men generally higher in comparison to white men. Quantitative determinations of serum HDL apoproteins from healthy men showed significantly lower apoA-I and apoA-II levels in smokers than in nonsmokers and the levels were negatively correlated with the number of cigarettes smoked per day.(29)

The Bogalusa Heart Study has provided lipoprotein profiles of a biracial pediatric population.(30) Age- and sex-related lipoprotein differences differ markedly from adult patterns and serum apolipoproteins demonstrate intrinsic metabolic differences among the race-sex groups, resulting in variability in lipoprotein composition, levels and atherogenic potential. (31) The distribution of lipids and lipoproteins in the children from families in which the father had angiographically proven coronary artery disease by 50 years of age bore a close resemblance to those observed in the affected fathers. (32) Therefore, genetic and environmental factors and their interactions play important roles in the age of onset and severity of ischemic heart disease.

Bodurtha reported, using the MCV twin population, that children with a family history of premature cardiovascular death had lower levels of HDL2-C than those without such a history.(33) White girls reporting a high level of physical activity had higher levels of HDL-C and HDL2-C than their more sedentary peers. In general children of mothers who smoked had lower HDL2-C than the children of nonsmoking mothers. Further evaluation of monozygotic and dizygotic twin pairs and their parents in the proposed study will provide further insight into the relationship between the genetic and environmental factors responsible for an increased risk of ischemic heart disease.

In summary, diverse changes in systemic oxygen delivery are noted in both active and passive smokers. We hypothesize that chronic passive cigarette smoking alters not only systemic oxygen delivery but other coronary heart disease risk factors. Since there is known familial aggregation of coronary heart disease risk factors, this study proposes to test the causal mechanisms and quantitate genetic and remedial environmental determinants of these mechanisms which may be responsible for the familial effect of premature coronary heart disease.

#### C. Preliminary Studies

The results of these preliminary studies have been presented at the American Heart Association Scientific Sessions. The abstract is included in Section 3. Appendix I.

From a population of 330 twin families enrolled in a Medical College of Virginia longitudinal cardiovascular disease study, 204 twin families were studied as a pilot project. Family members underwent anthropometric, cardiovascular, hematologic, pulmonary, hemorheologic and lipid pool evaluations. The twin sets are comprised of 53% monozygotic and 47%

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dizygotic pairs. There are 201 male and 207 female twins, mean age 11. years (range 11-12.5 years). Twenty-two percent of the twins are black. The mean age of the mothers (n=174) is 37.8 years and the mean age of the fathers (n=110) is 40.5 years. The incidence of twin passive smoking is 21% and no twin had a positive active smoking history. The fathers started smoking at  $17.6 \pm 5.6$  years of age and smoke  $25.6 \pm 12.9$  cigarettes/day. The mothers started smoking at  $18.5 \pm 4.6$  years of age and smoke  $18.5 \pm 10.9$  cigarettes/day. The results of the pilot study are presented in the following Tables.

Table 1. Body size and blood pressure data\*

	Father	Mother	F-Twins	M-Twins
NS(102)	S(34)	NS(134)	S(40)	NS(166)
BSA(m <sup>2</sup> )	2.06	2.01	1.79	1.76
BP-SYST(torr)	120	119	116	115
BP-DIAS(torr)	79	80	77	77
HT(cm)	176	178	163	163
WT(kg)	85.7	86.3	70.7	68.6
			PS(41)	NS(160) PS(41)
			1.28	1.25
			109	109
			61	62
			149	149
			40.1	42.6
				42.3

\*data represent mean values. F=female, M=male, NS=nonsmoker, S=smoker, PS=passive smoker. a) p<0.05

Twin height was related to maternal smoking. Twins of mothers smoking greater than 10 cigarettes/day were shorter than twins of mothers smoking less than 10 cigarettes/day ( $146.7 \pm 7.2$  vs  $143.9 \pm 6.9$ cm, p<.05). While systolic blood pressure was similar for passive smoking and non-smoking male and female twins, diastolic blood pressure was higher in the passive smoking male twins as compared to nonpassive smoking male twins.

As seen in Table 2, active smoking parents demonstrated significantly higher hematocrit and 2,3-DPG levels than nonsmoking parents. These changes were of a greater magnitude in the mothers. Passive smoking twins had hematocrit values that were similar to nonpassive smoking twins. The 2,3-DPG correlated with the SCN concentration, r=.28, p<.0001. These data suggest the existence of a certain degree of oxygen transport impairment which is compensated in part by increased levels of 2,3-DPG. The majority of twins were prepubertal or in early puberty with a paucity of steroid and adenohypophyseal hormones which are known to positively influence erythropoiesis.(20) Longitudinal evaluation of passive smoking and nonpassive smoking twins as they mature through puberty may detect developmental changes in erythropoiesis that do not presently exist and which may be related to the degree of cigarette smoke exposure.

Table 2. Hematologic and hemorheologic data\*

	Father	Mother	Twins
NS(98)	S(36)	NS(133)	S(42)
HCT	$43.8 \pm 2.7$	$45.1 \pm 2.5$ a	$39.9 \pm 2.4$
DPG	$2.24 \pm .36$	$2.44 \pm .33$ b	$2.09 \pm .29$
	Father	Mother	Twins
NS(79)	S(31)	NS(113)	S(33)
V-90sec-1	$5.2 \pm 0.7$	$5.4 \pm 0.9$	$4.6 \pm 0.7$
V-45sec-1	$6.0 \pm 0.9$	$6.2 \pm 1.0$	$5.2 \pm 0.9$
V-22sec-1	$7.4 \pm 1.3$	$7.5 \pm 1.5$	$6.2 \pm 1.3$
			NS(279) PS(62)
			$4.6 \pm 0.7$
			$4.5 \pm 0.8$
			$5.3 \pm 0.9$
			$5.1 \pm 1.6$
			$6.2 \pm 1.4$
			$6.1 \pm 1.6$

\* data represent mean  $\pm$ SD. abbreviations as in Table 1. HCT=hematocrit(%), DPG= 2,3 DPG (um/ml), V=viscosity (cPs). a) p<.05; b) p<.01; c) p<.001; d) p<.0001.

Healthy, middle-aged males have higher blood viscosity values than females which is a reflection of the higher hematocrit values in adult males and the relationship of viscosity to hematocrit ( $r=.60$ , p<.0001). Smoking parents have higher blood viscosity values than nonsmoking parents of the same sex through these did not reach statistical significance.

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These trends are consistent with existing data.(?) Viscosity values were similar in passive and nonpassive smoking twins.

Table 3. Erythropoietin data\*

	<u>Mother</u>	<u>Father</u>	<u>All Twins</u>			
EP	NS(10) 10.9±4.2	S(5) 9.8±5.4	NS(16) 10.9±3.2	S(7) 7.2±2.1 b	NS(21) 8.6±2.9	PS(10) 10.2±4.4
		<u>Male Twins</u>		<u>Female Twins</u>		
EP	NS(42) 8.7±3.4	PS(3) 7.0±2.0		NS(7) 8.4±1.9	PS(7) 11.5±4.5	

\* data represent mean ±SD. EP=erythropoietin (mIU/ml). b) p<0.01. A small number of serum erythropoietin levels have been obtained. Nonsmoking male and female adults and twins appear to have similar serum concentrations of erythropoietin with lower levels in twins. Smoking parents have lower erythropoietin levels than the nonsmoking parents of the same sex, though this reached significance only in the fathers (p<.01). Erythropoietin levels were higher in female passive smoking twins than in female nonpassive smoking twins. Inadequate numbers of individuals have been studied to date to make any conclusions. However, these data may be in agreement with recently published data, that an elevation in erythropoietin level in response to hypoxia will be transient if it results in a rise in hemoglobin concentration "appropriate" to the degree of hypoxia.(35) If the eventual rise in hemoglobin is appropriate, then the serum erythropoietin level returns to normal. Since the hematocrit levels were the same in passive and nonpassive smoking twins, the erythropoietic response to the passive smoking stress may be inadequate in the pre-or early-pubertal individual to return oxygen delivery to normal. Elevated erythropoietin levels may persist and other adaptive processes may be of greater importance in the young than in the adult. Longitudinal evaluation of erythropoietin levels and their interrelationships with other oxygen delivery variables in smoking and nonsmoking twins and their parents will provide further insight into our understanding of systemic oxygen transport in health and disease.

Table 4. Cigarette smoke exposure data\*

	<u>Father</u>	<u>Mother</u>	<u>Twins</u>
SCN	NS(42) 6.6±10.3	S(25) 15.8±7.2 d	NS(170) 2.4±3.8
COT	NS(9) ND range (27-619)	S(5) 330 c range (3-643)	PS(43) 8.3±4.0 d PS(21) 1.6 a range (1-9)

\*data represent mean ±SD. abbreviations and statistical significance as defined in Table 2. SCN=thiocyanate(mg/L), COT=cotinine(ng/ml). ND=not detected.

The serum SCN level was useful in separating active smokers from nonsmokers and discriminated between passive smoking and nonpassive smoking twins (p<.0001). There is conflicting data in the literature as to the usefulness of SCN in determining passive smoking exposure.(36-38) Although SCN has a longer half-life than cotinine (14 days vs 15-40 hours) and would therefore, be more likely to identify a smoker or passive smoker who has not been exposed to smoke for 8-12 hours, it is also derived from a number of dietary sources. In the present study, twin SCN level correlated with cotinine level ( $r=.52$ ,  $p<.0001$ ). Cotinine, a major metabolite of nicotine, is specific for tobacco, has a relatively long half-life and has been quantified in serum and urine of children.(39,40) Cotinine levels have been shown to correlate with the number of smokers in

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the home, the amount smoked by the mother, and the amount smoked by others in the home. Therefore, accurate measures of active and passive smoking are provided in the present study.

The preliminary results of the resting m-mode echocardiographic evaluation (shown in Table 5.) suggest that passive smoking male twins have thicker interventricular septa and LV posterior walls than nonpassive smoking male twins. The increased wall thicknesses were seen solely in the male passive smoking twins despite similar passive smoking exposure (SCN: males  $8.7 \pm 4.1$  vs females  $7.8 \pm 4.1$  mg/L; COT: males  $1.7 \pm 2.6$  vs females  $1.5 \pm 2.6$  ng/ml). Male twins generally had larger LV masses than female twins ( $p < .0001$ ) regardless of passive smoking status. Left ventricular relative wall thickness, defined as the ratio of LV radius to LV posterior wall thickness, was reduced in passive smoking male twins when compared to nonpassive smoking male twins ( $p < .01$ ). These data suggest that chronic passive smoking twins have increased LV wall thickness and mass which increase myocardial oxygen requirements. It is interesting that these changes are noted solely in males, who have a higher incidence of ischemic heart disease than females. Since twin 2,3-DPG is suggested to be increased in chronic passive smoking, altered oxygen supply/demand may be a mechanism for the increased LV thickness and the subsequent development of ischemic heart disease. Alternatively, these changes may be due to chronic exposure to carbon monoxide from cigarette smoke.(26)

While the echocardiographically determined cardiac index was similar for nonpassive smoking male and female twins, the determinants of the cardiac output—namely, heart rate and stroke volume—were different. The resting heart rate was higher and the stroke volume was smaller in females ( $p < .0001$ ). These changes likely reflect conditioning responses (males being less sedentary) as opposed to maturational differences as reflected in preliminary data in this population relating resting heart rate to self-reported activity and fitness.(33) In support of this hypothesis, the mean arterial pressure was found to be lower ( $73.5 \pm 8.3$  vs  $77.2 \pm 8.6$  torr,  $p < .0001$ ) and the pulse pressure wider ( $51.3 \pm 12.5$  vs  $47.6 \pm 13.3$  torr,  $p < .01$ ) in male twins. The calculated systolic wall stress index of the LV was lower in males ( $30.5 \pm 4.8$  vs  $31.7 \pm 5.1$  torr,  $p < .05$ ). Maturational differences in the determinants of cardiac output between male and female twins may become apparent during the analysis of the longitudinal data.

Table 5. Echocardiographic data\*

All Twins		F-Twins		M-Twins	
NS(298)	PS(81)	NS(155)	PS(41)	NS(140)	PS(40)
IVS	.64±.07	.64±.07	.64±.07	.65±.07	.68±.07 a
LVPW	.64±.07	.66±.07	.64±.08	.64±.06	.65±.07 b
LVMI	$64.2 \pm 10.4$	$66.2 \pm 10.8$	$61.6 \pm 9.7$	$62.5 \pm 9.4$	$67.2 \pm 10.4$ $70.0 \pm 9.4$
R/TH	$3.53 \pm 1.43$	$3.44 \pm .39$	$3.48 \pm .46$	$3.49 \pm .40$	$3.59 \pm .39$ $3.39 \pm .39$ b
HR	$73 \pm 12$	$70 \pm 10$	$77 \pm 13$	$75 \pm 10$	$69 \pm 9$ $65 \pm 8$ b
SV	$38 \pm 7$	$38 \pm 6$	$36 \pm 6$	$37 \pm 7$	$40 \pm 7$ $40 \pm 5$
CI	$27.9 \pm .59$	$2.72 \pm .61$	$2.80 \pm .59$	$2.83 \pm .77$	$2.79 \pm .58$ $2.61 \pm .38$ a

\* data represent mean SD. abbreviations as defined in Table 1 & 2.  
 IVS=interventricular septum(cm), LV=left ventricle, PW=posterior wall(cm),  
 MI=mass index(g/m<sup>2</sup>), R/TH=relative wall thickness, HR=heart rate(bpm),  
 SV=stroke volume(ml), CI=cardiac index((L/m/m<sup>2</sup>). P values as previously  
 defined.

Table 6. Parental lipid and lipoprotein data\*

	Father	Mother
NS(80)	S(19)	S(118)
CHOLESTEROL(mg%)	$216 \pm 41$	$201 \pm 35$
LDL(mg%)	$114 \pm 29$	$105 \pm 24$
HDL(mg%)	$42 \pm 10$	$38 \pm 9$ a
HDL2(mg%)	$8 \pm 6$	$6 \pm 4$ a
		$55 \pm 13$
		$16 \pm 11$
		$42 \pm 9$ d
		$9 \pm 4$ d

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\*Number pages consecutively at the bottom throughout the application. Do not use suffixes such as 1a, 2a.

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The preliminary lipid and lipoprotein data in the parents are in agreement with existing literature. Generally, women have higher HDL-C and HDL2-C levels than men. Decreased levels of these lipoprotein fractions are seen in the smoking parents when compared with the nonsmoking parent of the same sex. An inverse relationship was found between HDL-C and SCN in the fathers ( $r=-.46$ ,  $p<.001$ ).

**Table 7. Twin lipid and lipoprotein data\***

All Twins		F-Twins		M-Twins	
NS(289)	PS(70)	NS(148)	PS(30)	NS(139)	PS(40)
HDL	49±10	46±8 a	46±9	48±8	50±10
HDL2	14±8	12±6 b	14±8	14±6	15±9
					11±5 c

\* data represent mean SD. abbreviations and statistical significance as defined previously. All units are mg%.

The HDL and HDL2 lipoprotein subfraction levels were lower in passive smoking male twins than in the nonpassive smoking male twin. In general, twins of smoking mothers had lower levels of HDL2 than the twins of nonsmoking mothers ( $11.8\pm6.0$  vs  $14.4\pm7.0$  mg%),  $p<.05$ ). A similar trend was seen in children of smoking fathers ( $12.6\pm7.8$  vs  $14.6\pm8.0$  mg%). It appears therefore, that parental smoking may contribute to changes in this risk factor in the male children as well in the smokers themselves. However, the number of covariates which have been shown to influence HDL-C and its subfractions is large.(33) Age, race, sex, body habitus, fitness, lifestyle variables and genetic background are all known or suspected to have an effect on these variables. A more detailed analysis and a larger sample will be required to delineate the contributions of genetic and environmental influences on serum lipid and lipoprotein levels. With the availability of longitudinal data on twins and their parents, an analysis will be possible of these influences on the developmental changes that occur during adolescence in serum lipid and lipoprotein levels.

**Table 8. Pulmonary Test data\***

Parents		Twins	
NS(51)	S(16)	NS(111)	PS(18)
R-FVC (L)	3.72±.79	3.95±1.0	2.70±.65
E-FVC (L)	3.81±.81	4.07±.97	2.71±.63
R-FEV1 (L)	3.22±.67	3.32±.81	2.41±.53
E-FEV1 (L)	3.30±.72	3.31±.81	2.39±.53
R-EF25-75% (L/s)	3.96±1.12	3.56±1.01	2.88±.69
E-EF25-75% (L/s)	4.13±1.25	3.58±1.11	2.76±.76
R-EF75-85% (L/s)	1.46±.49	1.24±.47	1.38±.47
E-EF75-85% (L/s)	1.50±.50	1.35±.60	1.36±.55
R-FEF 50% (L/s)	4.48±1.25	4.03±1.08	3.13±.76
E-FEF 50% (L/s)	4.61±1.43	3.82±1.30 a	2.9 ±.83
			2.83±.91

\* data represent group mean SD. abbreviations and statistical significance as previously defined. R=resting, E=postexercise, FVC=forced vital capacity, FEV1=forced expiratory volume in 1.0 second, EF=expiratory flow, FEF=forced expiratory flow rate.

Pulmonary function testing has been performed on family members before and 15 minutes following exercise termination, to coincide with the peak bronchospastic response to exercise. In the small number of parents studied, resting and exercise FVC and FEV1 were similar in smoking and nonsmoking individuals. Evidence for increased small airway resistance is suggested by lower forced expiratory volumes at 25-75% and 75-85%. Statistical significance was not reached for these differences, possibly due to the small number of smokers. The percent of predicted FEF50% was lower in smoking parents ( $90.2\%$  vs  $108.8\%$ ,  $p=.01$ ) as was the percent of predicted peak expiratory flow rate ( $88.2\%$  vs  $96.5\%$ ).

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Passive smoking twins demonstrated slight reductions in FVC and FEV<sub>1</sub> with no significant differences in FEF25-75%, FEF75-85% and FEF50% when compared to nonpassive smoking twins. Only a small number (n=18) of passive smoking twins have been studied. However, passive smoking twins had almost a three fold greater number of asthmatic episodes when compared to nonpassive smoking twins ( $p<.005$ ). The effects of parental smoking on childhood pulmonary function abnormalities may be cumulative, as older children demonstrate greater abnormalities. (41) The role of genetic and developmental factors modifying small airway function is not clear, although effects of genetic factors are known to be present. Further investigation of mono- and dizygotic passive and nonpassive smoking twins and their parents are indicated and will provide new, useful information.

No significant differences were observed between active and nonsmoking adults or between passive and nonpassive smoking twins in peak bicycle exercise capacity, heart rate increase, or blood pressure changes during exercise testing. However, the decrease in the slope of the diastolic blood pressure with exercise was less steep in twins of mothers smoking greater than 10 cigarettes/day ( $-1.54 \pm 1.19$  vs  $-2.06 \pm 1.47$ ,  $p<.05$ ). This suggests that passive smoking children have less reactivity to their arterial vascular bed with an altered ability to vasodilate when compared to nonpassive smoking children. The ability to detect actual differences between groups may have been hampered by the submaximal nature of many of the exercise performances. Standardization of each stage of exercise with measured oxygen consumption would permit a complete and more accurate analysis of exercise responses.

Table 9. Family Correlations for Blood Pressure and Heart Rate

--	Variable	Spousal	Parent-Twin	DZ	MZ
	SBP	.05	.05	.23	.69
	DBP	.08	.08	.27	.49
	HR	.10	.26	.36	.72
n(pairs)		121	698	104	122

For all the measures in Table 9., the correlation for MZ twins is significantly higher than for DZ twins. This observation confirms that for 11 year olds that genetic effects play a major role in creating individual differences in cardiovascular measures. The MZ correlation is twice the DZ correlation (exactly for HR and within sampling error for SBP and DBP) as one would expect if mating is random with respect to the causes of juvenile measures, gene action is additive, and family environment does not cause twin resemblance. If the same genes are affecting adult and juvenile measures we would estimate that the parent-offspring correlation should equal the DZ twin correlation since both share half their genes in common(66) This is not the case for SBP or DBP for which the parent-offspring correlation is virtually zero- compelling evidence that the genetic control of juvenile blood pressure is different from the control of adult blood pressure.

The results for HR are strikingly different. The parent-offspring correlation is highly significant and much closer to the DZ correlation. This means that adult HR is largely controlled by the same genes as HR in 11 year olds. The parent-offspring correlation is still significantly lower than the juvenile twin data would predict. There are two possible reasons for this finding. (66) Either there are still some age-specific genetic effects, i.e. not all the genes expressed in juveniles are still expressed in adults, or the genetic effects in juveniles have still to reach their adult magnitude. These findings provide a strong case for the longitudinal study because we need to analyze the genetics of these and other variables in older subjects so that we can not only find the age at which "adult" genetic effects begin to stabilize but also to test for

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important genotype environmental interactions; Are some genotypes particularly susceptible to the effects of passive smoking, or does passive smoking either enhance or suppress the expression of normal genetic variation in the determinants of systemic oxygen delivery and possibly increase the risk for disease production.

Table 10. Twin BP and HR Correlations by Sex and Zygosity

	SBP	DBP	HR	N (PAIRS)
MZ PAIRS:M-M	0.74	0.56	0.71	56
MZ PAIRS:F-F	0.65	0.44	0.67	68
DZ PAIRS:M-M	0.37	0.50	0.53	22
DZ PAIRS:F-F	0.38	0.36	0.07	29
DZ PAIRS:M-F	0.08	0.07	0.44	31

The SBP and DBP data on the 11 year old twins also provide evidence of sex differences in the genetic control of blood pressure. For both SBP and DBP the correlations for unlike-sex DZ twins is zero and significantly less than the correlation for like-sex DZ's. This means that the control of blood pressure is different in the two sexes at this age. These results indicate that the effects of genes and environment are changing at puberty. Analysis of longitudinal data on these and other variables should provide new and interesting information on the genetic control of systemic oxygen delivery.

Table 11. Heart Rate Correlations Across Visits

MZ CORRELATIONS	UPPER TRIANGLE: N Vis-1=126 Vis-2=61				
DZ CORRELATIONS	LOWER TRIANGLE: N Vis-1=107 Vis-2=45				
	Twin 1		Twin 2		
Twin 1	Vis1	Vis2	Vis1	Vis2	
	Vis1	1.00	0.54	0.64	0.47
Twin 2	Vis2	0.64	1.00	0.41	0.65
	Vis1	0.31	0.37	1.00	0.73
Vis2	0.23	0.43	0.64	1.00	

Table 11. gives the cross-occasion (18 months apart) cross-twin correlations for heart rate in MZ and DZ twins. These correlations illustrate three main principles which will be extended across the period of puberty into adolescence when the full data set is available. First, within each occasion the correlations for MZ are greater than those for DZ twins. Thus genetic factors affect heart rate at both ages. Second, the correlation of the first and second twin across occasions is large, but smaller than the correlation within occasions. This occurs because different factors influence heart rate on the two occasions. Third, the cross-twin cross occasion correlations are greater for MZ than DZ twins, confirming that there are some genetic effects which exert a long-term influence on heart rate. Taken together, these three findings suggest that some genes exert a long term effect on heart rate and other genetic effects are relatively short term and specific to the particular ages at which the measurements were taken.

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D. Experimental Design and Methods  
a. Study Design

The unique aspects of this study are 1) the twin-parent design which allows us to test more subtle genetic and environmental hypotheses than is possible with nuclear families or twins alone; 2) the longitudinal component which permits the first rigorous analysis of the developmental changes in the genetic expression on the hematologic and cardiovascular determinants of systemic oxygen delivery; 3) the structure and size of the sample which is adequate to test the developmental hypotheses and 4) the inclusion of unlike sex twins to permit the analysis of the consistency of genetic and environmental effects across the sexes. By integrating the longitudinal and cross-sectional components, the proposed design will permit a unique and critical evaluation of the genetic control of systemic oxygen delivery and the effects of childhood passive smoking on cardiovascular and pulmonary disease risk that could not be answered by either study alone.

b. Power

There are two principal concerns about the power of this study: 1) will the number of subjects in smoking and non-smoking families be sufficient to detect the effects of passive smoking? 2) will the number of twin pairs be sufficient for genetic and environmental model fitting?

From standard power analyses (42), if 120 subjects from smoking families and 480 subjects from non-smoking families are studied there will be a 95% chance of detecting as statistically significant a passive smoking effect that accounts for 9% of variance, and more likely than not to detect as significant at the 5% level an effect which accounts for as little as 3% of variance. This is for a cross-sectional analysis at only one age. Combining the repeated measurements gives considerable additional power, the exact extent of which depends on the breakdown of the other components of variation.

Stratification of groups by zygosity, sex, and smoking status will decrease the size of each group. Fifty-three percent of the twin pairs enrolled in the pilot study were monozygotic twins and 51% of all twins were female. For the proposed evaluation of 300 sets of twins, the group of monozygotic female passive smoking twin pairs could be as small as 16 pairs or 32 individuals. Enrolling 32 individuals in each group will insure a sample size sufficient to detect intergroup differences. For example, a key variable which is easily measured is the LV posterior wall thickness. The normal distribution of this variable is 0.6 to 1.2 cm and a 0.3 cm change would be considered significant. An acceptable standard deviation of error for this measurement is 0.03 cm. (43) Using 32 subjects, the power of the test would exceed .99 or, there is better than a 99% probability that the test will be able to detect a 0.3 cm difference between groups. The power will actually be even greater than this as repeated measures will be made.

Power calculations for the twin analyses (44) confirm that with 300 twin pairs a hypothesis of no genetic variation can be rejected with 95% power at the 5% level when as much as 70% of the variation is attributable to random environmental effects. The presence of correlated family environments reduces the power of the test for genetic effects, but securing repeated measurements of many of the variables increases it. Many of the hypotheses addressed do not depend on information from a single variable measured on one occasion, but on several variables measured longitudinally. Finally, the sample consists not only of twins but includes the parents of the juvenile twins who will provide additional information about the contribution of additive genetic and environmental factors. We are therefore confident that the study has the power to resolve the most important competing hypotheses.

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**c. Ascertainment of twins**

Twins for the proposed study have been ascertained from the middle schools of Virginia with use of a computerized population-based registry. Approximately 400 pairs of twins will be readily available for the study. From these, a total of 330 pairs will be recruited for the study. The families of twins meeting the age and geographic criteria for inclusion are contacted initially by the school to maintain confidentiality from the investigators. The parents reply by mail and after a telephone follow up, are invited to complete a zygosity questionnaire. Three cohorts of 100 eleven year old twins have been enrolled. During each of three testing cycles, a new cohort is added to those previously studied. To allow for attrition, 110% of the desired number of pairs are studied. Therefore, for the purposes of the proposed study, 330 sets of twins will be evaluated prospectively and longitudinally.

**d. Protocol**

**1. Overview**

The following data will be collected on parents and their offspring in the initial testing cycle; 1) genotyping; 2) health questionnaire; 3) anthropometric measures; 4) resting hemodynamic measures; 5) dynamic exercise measures; 6) hematologic and hemorheologic measures; 7) echocardiographic left ventricular measures (twins only); 8) pre- and post-exercise pulmonary flow/volume loops; and 9) lipid profile. During the following two testing cycles, subjects will have all tests repeated except genotyping and the questionnaire.

**2. Time Table**

After full oral description of its longitudinal nature and the tests to be performed, families will then be scheduled for the first of the three 18 month testing cycles of longitudinal testing. Follow up testing during the second and third cycle will be scheduled within two weeks of the sesquiannual anniversary of testing in the first cycle. The parents of the twins will be studied only during the first two test periods for each cohort, and will have their height, weight, skinfold thickness, right arm seated blood pressure and heart rate measured. Pulmonary flow/volume loops will be recorded prior to exercise and 15 minutes following exercise. The parents will also undergo exercise on a bicycle ergometer with oxygen consumption measurements which will be monitored by trained staff.

Blood samples will be obtained before exercise or after an adequate rest period following exercise. The parents will provide questionnaire data on family history of heart disease and potential cardiovascular risk factors such as cigarette use, the number of cigarettes smoked per day, duration of cigarette use and alcohol use. Similar anthropometric, cardiovascular, pulmonary, hemorheologic and hematologic measurements will be obtained from the twins at each of the three test cycles. In addition, the stage of puberty will be scored using the Tanner Scale, left ventricular dimensions and function will be measured by echocardiography and the twins cardiovascular responses to dynamic exercise will be determined.

**3. Measurements**

a) Zygosity determination- Zygosity will be assessed initially by a questionnaire confirmed by dermatoglyphic analysis and testing twins and their parents, whenever possible, for the ABO, MNS, Rh, Kell, Fy, Hp, Tf, Hb, PGM, AP, G-6-PD, 6-GD, Ct, and LDH systems. With this battery of polymorphisms, the probability of dizygosity for concordant pairs typically is less than 0.001.(45)

b) Anthropometric Measurements-

(i) Height of each subject in stocking feet will be measured with an anthropometric plane and square. The height will be measured to the nearest 1/10 centimeter.

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(ii) Weight will be measured to the nearest 1/10 kilogram, where the subject is wearing indoor clothing and is shoeless on a digital scale. Every three months, the scales will be cleaned and checked for accuracy using certified weights by a scale servicing company. Each day prior to weighing of the subjects, the scale is balanced and adjusted if necessary.

(iii) Skinfold thicknesses in the biceps, subscapular, triceps, suprailiac and calf are measured using Holtain calipers according to the method of Marshall and Tanner.

(iv) Sexual maturation will be assessed by the research associate during the collection of the echocardiographic data, using the stages developed by Marshall and Tanner.(46,47)

c) Cardiovascular Measurements

(i) Resting Blood pressure will be measured using a Zero Muddler Instrument to minimize digit preference. The device, a random-zero common sphygmomanometer, enters a random number for the zero level. The person measuring the blood pressure is unaware of the actual measurement while taking the blood pressure. Blood pressure is measured in the sitting position with the appropriate compression cuffs; pediatric, adult, obese, and thigh. The chosen cuff will be big enough to circle at least half of the upper arm without overlapping. The rubber bladder rests over the artery being compressed, and it has sufficient width to cover at least two thirds of the upper arm. The pressure within the pressure cuff indicated by the level of mercury column at the murmur at the first, fourth and fifth phase Korotkoff sounds will be noted and recorded. The blood pressure is recorded three times and initialed by the observer while the pulse rate will be measured with a cardiotachometer.

(ii) Echocardiograms will be obtained in the recumbent position using an SKI ultrasonoscope 20A with a 3.5 MHz transducer and Honeywell 1856 strip chart recorder. The "standard interspace" techniques for transducer position will be utilized. The ultrasonic beam will be angulated toward the apex of the left ventricle to record the maximal dimension showing both rapid systolic posterior left ventricular septal motion and rapid anterior left ventricular posterior wall motion. Left ventricular dimensions and wall thicknesses will be measured in a standard fashion.(48) Systolic and diastolic LV volumes will be calculated by the method of Teichholz.(49) An echocardiographic stroke volume will be calculated by: Stroke volume (SV)=LV diastolic volume- LV systolic volume. Cardiac output (CO)=SV X heart rate. Left ventricular mass will be calculated using a standard formula.(50) Left ventricular relative wall thickness (R/TH) is expressed as the end-diastolic chamber radius to wall thickness ratio.(51)

(iii) Dynamic exercise testing will be performed with a bicycle ergometer that is modified to provide a special low saddle with adjustable handle bars and paddle crank for children. The bicycle ergometer is calibrated by the manufacturer to indicate the workload (actual power output in Watts). The subject will be asked to cycle on the electronically braked ergometer which maintains a given power output within range of 60-80 rpm. Heart rate will be measured with an electrocardiogram, and blood pressure measured by an automated system (Critikon). Oxygen consumption will be measured continuously by a flow through face mask system (Waters MRM-1). The measurements will be done at rest to accustom the subject to the apparatus. The subject will begin to cycle at a workload of 25 Watts. The workload will be increased every 2 minutes by a fixed amount depending upon height, e.g., for children between 125-150cm, the increment is 15 Watts. The subject will be encouraged to continue exercising either until physical and mental fatigue supervene or the heart rate reaches 170 beats per minute. Oxygen consumption will be recorded at each exercising workload. Blood pressure and heart rate will be monitored and recorded every two minutes for six minutes during recovery phase of the testing.

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d) Hematologic and Hemorheologic Measurements

(i) Hematocrit - Venous blood obtained by venipuncture will be collected in a Vacutainer (Becton, Dickinson Co. Inc.) containing 0.04 ml. of powdered EDTA. Two heparinized microcapillary tubes will be filled with the collected blood and centrifuged at 10,000 rpm for five minutes and the hematocrit of duplicate samples will be read directly and averaged.

(ii) 2,3-DPG Assay - One ml of freshly drawn blood is accurately added to 3.0 ml of cold trichloroacetic acid solution (8% w/v) and shaken vigorously. The samples are then centrifuged to obtain a clear supernatant. The concentration of 2,3-DPG in the supernatant is determined by a quantitative colorimetric method at 660nm. Phosphorus, produced by the enzymatic hydrolysis of 2,3-DPG, is determined by the method of Fiske and SubbaRow and is equivalent on a molar basis to the 2,3-DPG present.(52,53)

(iii) Thiocyanate Assay - One ml of freshly drawn blood is accurately added to 5.0 ml of trichloroacetic acid solution (8% w/v) and shaken vigorously. The samples are then centrifuged to obtain a clear supernatant. The concentration of thiocyanate in the supernatant is determined by a quantitative colorimetric method at 460nm. (54,55)

(iv) Cotinine Assay - Serum cotinine levels are performed by the Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, NY (Daniel Sepkovic, Ph.D., Senior Associate, Section of Clinical Biochemistry). The concentration of cotinine is quantitated using radioimmunoassay (RIA) methods.(56,57) Written agreement and cost structure are provided in Section 3. Appendix II.

(v) Erythropoietin Assay - Serum erythropoietin levels are performed by Smith Kline Bio-Science Laboratories, Van Nuys, CA(C. Dennis Ashby, Ph.D., Director, Department of Endocrinology/RIA). The concentration of erythropoietin is quantitated using RIA methods.(21) Written agreement and cost structure are provided in Section 3. Appendix II.

(vi) Viscosity Profile - Viscosity of whole blood (at 37 C) is determined with a cone plate digital viscometer (Brookfield Engineering Laboratories, Inc.) utilizing a 0.8 cone and 0.5cc of whole blood. Viscosity is measured at shear rates from 22 sec<sup>-1</sup> to 90sec<sup>-1</sup>. The blood sample is gently aspirated with a needleless tuberculin syringe to avoid hemolysis from shear forces.

e) Lipid Profile

Comprehensive plasma lipoprotein levels and profiles are performed by the Lipoprotein Laboratory at the University of Alabama Medical Center (Jere P. Segrest, M.D., Ph.D, Director). Levels of total cholesterol, triglycerides, Lipoprotein a, LDL, HDL (HDL-2 and -3), VLDL and IDL are measured by a vertical auto profile analysis method.(58)

f) Pulmonary Flow/Volume Loops

Spirometry and flow volume loops will be performed at rest and 15 minutes following exercise using the MultiSPIRO-PC Spirometry System. Air flow is measured by a pneumotachometer. Measurements representing the best effort of three trials will be recorded. In addition to flow/volume loops, the system provides measures of FVC, FEV 0.5, FEV 1, PEF, FEF .2-.1.2, FEF 25-75%, FEF 75-85%, FEF 50%, FIV 1, PIF and FIF 50%.

4. Quality Control

In each new family unit that is studied, one of the parents or twins will be randomly selected for replicate echocardiographic testing. The subject selected will be studied first, and all family members will then be studied in a random sequence. Comparison of replicate determination will permit estimation and monitoring of the measurement error. Reproducibility data to date demonstrate significant correlations between the first and second echocardiographic examination on the same day (N=68; interventricular septum r=.88, LV diastolic dimension r=.98, LV systolic dimension r=.97, LV posterior wall r=.86; all p<.001) An analysis of data

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collected in different families on the same day would permit the detection of any temporal trends in the test procedure. Calibration curves and standards for the 2,3-DPG and thiocyanate assays are measured in temporal relationship to sample measurements. The MultiSPIRO-PC and viscometer are calibrated each day prior to their use.

#### 5. Data Management

The Cardiovascular Twin Data system currently contains data on up to three visits of three cohorts of twins and their parents participating in the MCV Twin BP study. A subgroup of these subjects form the present study population. The dataset resides on a direct access device on the university's IBM mainframe system. The data set is in the form of a SAS (Statistical Analysis System) statistical procedures and the SAS programming language. (59) The file is backed up to tape and password protected to prevent inadvertent or intentional data losses.

While all of the datasets ultimately reside on the university's IBM mainframe computer, a particular dataset may follow one of several pathways. Several datasets exist on data collection sheets and are entered onto floppy diskette via an IBM microcomputer and dBASE III. Using dial-up modems, these files are subsequently transmitted over telephone lines to the IBM mainframe. The communications program Smarterm 125 by Persoft is used to accomplish this transfer. Other datasets are entered from data entry sheets onto a VAX system through a directly connected terminal. Verification is by double entry. VAX utility programs allow these datasets to be copied over to the IBM periodically.

The echocardiographic tracings are placed over a bit pad. A BASIC program on an IBM microcomputer processes output from the bit pad, calculates echocardiographic dimensions, and stores the information on diskette. The diskette data are transferred using Smarterm 125 to the VAX over telephone lines via modems. Using a VAX utility, the data are copied to the IBM mainframe where the echocardiographically derived variables such as cardiac output and left ventricular mass are calculated.

To ensure that no data are lost during the transfer of information to the IBM mainframe, logs are kept in which the number of lines in the original files are compared with the number of lines in the destination files on the IBM. Any discrepancy between the number of lines in the files is investigated and reconciled.

Edit programs were written for each dataset. These programs serve two purposes. They are used to perform edit checks on the raw data and they convert the data from raw files to SAS datasets. The data are checked for response validity, that is, inappropriate discrete responses and out of range numeric responses are detected, flagged and subsequently corrected. Some advantages of storing the data in SAS form are: 1) the data are ready for fast processing by statistical procedures in SAS, like means, frequency, t test, analysis of variance, regression, as well as the display procedures such as print, plot, and chart; 2) the data can be readily sorted, subset, or otherwise manipulated using the flexible SAS programming language; and 3) the contents of the datasets are well organized and documented by the contents procedure of SAS.

In many instances it is desirable to compare information from different datasets. To do this efficiently, a SAS data set is created consisting of the variables from all the separate datasets, merged by individual and visit. That is, all the information from a particular individual on a particular visit appears in a single record. From this large dataset temporary work datasets can be created. These work datasets can contain subsets of the variables or observations in the larger dataset. For example, a dataset with just systolic blood pressure measures and resting heart rate could be created. In addition, this dataset could be subset so that only twin boys, or mothers of like-sexed dizygotic twins were included. Whenever the merged dataset is created, a tape backup of

the entire system is made. That is, all of the separate datasets as well as the merged dataset are copied to tape. The copies are in SAS format, so that if the disk-based versions were damaged or deleted, they could be quickly restored from tape. The main dataset does not contain personal identifying information. The existence of the merged dataset has proven to be helpful in expediting the analysis of the data.

## 6. Data Analysis

### a. Introduction

This project's longitudinal twin family design, cross-classified with the presence or absence of a measurable environmental risk factor, leads to tests of genetic and environmental hypotheses which go beyond those considered in traditional epidemiological studies. The richness of the measurement protocol, ranging from laboratory observations of hemodynamics through to anthropometric measurements, provides the opportunity to test both univariate and multivariate hypotheses; this latter is particularly important since 1) it is unlikely that variation in the oxygen delivery system can be described without taking account of the interrelationships of its various components, and 2) a multivariate approach will provide additional power to detect what may be initially relatively small measurable effects.

Table 6.1 Outline of the experimental design.

SMOKING STATUS	ZYGOSITY	AGE AT TESTING (YRS)		
		11.0	12.5	14.0
PASSIVE SMOKER	MZ FAMILY 1	TWIN 1		
		TWIN 2		
	FAMILY 2	TWIN 1		
		TWIN 2		
	DZ FAMILY 31	TWIN 1		
		TWIN 2		
	FAMILY 60	TWIN 1		
		TWIN 2		
	MZ FAMILY 61	TWIN 1		
		TWIN 2		
NON-SMOKER	MZ FAMILY 180	TWIN 1		
		TWIN 2		
	DZ FAMILY 181	TWIN 1		
		TWIN 2		
	FAMILY 300	TWIN 1		
		TWIN 2		

The essential features of the study design are recapitulated in Table 6.1 where it can be seen that it has three key features; families fall into one of two risk categories according to the presence or absence of smoking in the parents; the twin pairs are either monozygotic (MZ) or dizygotic (DZ); and observations are taken at three successive ages. These features of the design respectively permit us to test for the effects of passive smoking, to examine the extent of genetic influences and to study developmental changes.

### b. Preliminary data cleaning, reduction and transformation

Before any analyses can be undertaken with a large data set of this kind, it is important that the raw data be thoroughly scrutinized for errors and omissions. The data management process, outlined in Section 2.D.5, has built in data verification checks. However, the resulting mainframe data files (e.g. those maintained as SAS files) are then subjected to a series of further checks for outliers and anomalous values using frequency counts and crosstabulations (e.g. using PROC FREQ in SAS).

Initial data reduction produces summary means and standard deviations for inspection. As a starting point for the analyses of variance, regression analyses and for model fitting to variance-covariance matrices, the data are structured in two alternative ways. For some analyses (e.g.

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analysis of variance and non-genetic regression analyses) the data are arranged with individuals as the cases (tagged by family membership etc.); this facilitates standard between and within group analyses of variance where the sums of squares are partitioned appropriately, or regression of dependent variables on independent or predictor variables where both sums of squares and sums of products are the required data summary. For genetic and environmental model fitting it is more convenient to structure the data using the family as the case with twin 1, twin 2, mother and father scores as different variables within the case; the natural data summary is now the variance-covariance matrix between the variables/individuals within the case and these are typically computed separately for zygosity and sex groups. Since both types of analyses are employed, both types of data structure files are maintained and both types of data summary are available.

The most powerful and flexible statistical procedures we wish to use make distributional assumptions which need to be at least approximated since, although many procedures are robust against departures from these assumptions, our tests and parameter estimates will be biased to a small extent by failure of these assumptions. We can examine the data for two types of problems. First the within group raw score distributions are inspected for departures from normality; this is done either visually or by computation of the distribution's third and higher moments. Second a test for heteroscedasticity is employed which capitalizes on the use of MZ twins and repeated measurements; for example, the polynomial regression of MZ absolute twin pair difference on twin pair mean will detect dependency of within family environmental variance (including errors of measurement) on genotypic and shared family environment score. Given either non-normality or heteroscedasticity, transformations of the raw data (e.g. logarithmic, square root or arcsine depending on the type of scale) can usually be found so that the assumptions of the statistical test are met.

#### c. Detecting mean effects of passive smoking: ANOVA

The test for the mean effects of passive smoking on quantitative variables (such as hemodynamic variables) is essentially a t test of the group mean difference against its standard error in the univariate case and Hotelling's T<sup>2</sup> in the multivariate case. However, the inclusion of "replicate" subjects within families, i.e. twins, and repeated observations at three different ages permits a fuller analysis of variance which is outlined in Table 6.2

Table 6.2 The structure of the epidemiological ANOVA.

Source of variation	df	expected mean squares
Between families	299	$\sigma_e^2 + \sigma_{\text{cat}}^2 + \sigma_{\text{cat} \times \text{family}}$
Passive smoking category(C)	2	$\sigma_e^2 + \sigma_{\text{cat}}^2 + \sigma_{\text{cat} \times \text{family}}$
Between families within category(S)	298	$\sigma_e^2 + \sigma_{\text{cat}}^2 + \sigma_{\text{cat} \times \text{family}}$
Within families (W)	300	$\sigma_e^2 + \sigma_{\text{cat}}^2$
Ages (A)	2	$\sigma_e^2 + \sigma_{\text{cat}}^2 + \sigma_{\text{cat} \times \text{age}}$
Ages X Between families	598	
A X C	2	$\sigma_e^2 + \sigma_{\text{cat}}^2 + \sigma_{\text{cat} \times \text{age}}$
A X S	596	$\sigma_e^2 + \sigma_{\text{cat}}^2 + \sigma_{\text{cat} \times \text{age}}$
A X W	600	$\sigma_e^2$

Where  $\sigma_e^2$  = random error  
 $\sigma_{\text{cat}}^2$  = variation within families  
 $\sigma_{\text{cat}}$  = variation between families within categories  
 $\sigma_{\text{cat} \times \text{family}}$  = effects of passive smoking  
 $\sigma_{\text{cat}}$  = mean differences between ages  
 $\sigma_{\text{cat} \times \text{age}}$  = differences between ages on effects of passive smoking  
 $\sigma_{\text{cat} \times \text{family}}$  = age differences in between family effects

$\sigma_{\text{cat}}^2 = 36$  where  $N=60$ ,  $M=240$ ,  $N=1100$

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In this table we recognize the familial correlations between the subjects and separate components of variation between ( $\sigma^2_B$ ) and within ( $\sigma^2_W$ ) families within smoking categories. The basic test for passive smoking effects is now the univariate F-ratio of mean square (C) against the mean square (B), or a multivariate analog, e.g. Wilk's lambda. Tests for the change (increase) with age of the effects of passive smoking (hypotheses A.2 and A.3) are similarly given by the ratio of mean square (A X C) to mean square (A X B). In setting out the expected mean squares in Table 6.2 we have assumed that both smoking category and mean age effects are "fixed" effects and that variation with age in within family effects is a measure of random error. Although our cell frequencies will be very closely proportional to each other (the only major departure being a result of the 20%-80% split between the smoking categories), we will perform these analyses using GLM rather than ANOVA in SAS so that we can readily incorporate additional factors such as sex. Given the potential complexity of the analysis, especially in the multivariate case, we may in practice perform the analysis of scores averaged over ages (the top half of Table 6.2), or cross-sectionally at any given age, separately from those of the interaction with age.

Perusal of the expected mean squares given in the table reveals the considerable additional power that is given to the study by including twins and repeated measurements. The occasion to occasion reliability of hemodynamic measurements, for example, at the age of the subjects is not as high as for young adults and "random error" may account for as much as 30-40% of the observed variation. By obtaining six distinct measurements for each family (two twins at 3 ages) we reduce the effective contribution of random error variation by a factor of six for testing the effects of passive smoking. The inclusion of twins similarly halves the effective contribution of within family sampling variation.

The analysis of variance approach is conceptually attractive and widely accepted and understood. It provides one approach to direct tests of hypotheses A.2, A.3, A.4 and specific goals A.1, A.2, A.3, A.5, A.7, A.8 and A.10.

#### d. Adjusting for the effects of covariates: ANCOVA

A natural extension of the analysis of variance allows us to consider the effects of treatment or subject variables after some adjustment has been made for the influence of other variables referred to as covariates. In particular, for the analyses described in Section 2.D.6.b we will want to consider the effects of passive smoking on outcome variables after adjusting for differences in such anthropometric measures as body weight. Does passive smoking affect left ventricular wall thickness after we have taken account of body weight? This is a statistical matching procedure for the anthropometric measures.

The appropriate procedure for making such adjustments is the analysis of covariance, which adjusts the between and within group variation by the linear regression of the dependent variable on the covariate. It should be noted that this procedure may in some circumstances reduce the within group variation proportionately more than that of between group and, hence, lead to the discovery of significant between group effects which were previously masked by within group variation associated with the covariate. Analyses of covariance (ANCOVA) need careful interpretation and will be used in conjunction with the linear structural modelling outlined in Section 2.D.6.g. The ANCOVA procedures are most readily implemented using PROC GLM in SAS. As an extension of the ANOVA they will provide direct tests of specific goals A.1, A.5, A.6, A.7 and contributor evaluations for specific goal A.2.

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e. Multivariate correlation and factor analyses

With so many measures available for analysis, we require a rational procedure for selecting variables or for combining measures into composite variables. The techniques of factor analysis may be used for this purpose. Subjects will be treated as individuals and compute the intercorrelations between variables of a given type. If the variables may be summarized effectively by one or a few factors, then factor analysis of the intercorrelation matrix will result in almost all of the observed variance being accounted for by these one or few factors; in mathematical terms the dominant eigenvalue/s of the matrix will approach the trace of the matrix in size. Given this situation the factor loadings may be inspected to identify individual variables which best measure the factor/s, or alternatively, factor scores may be computed and used for subsequent analysis. Factor analyses are readily implemented using PROC FACTOR in SAS which offers a variety of factoring procedures and output options including the output of factor scores. One advantage of using twins is that replicate data sets may be created each consisting of one twin from each pair. By running the analyses separately for each group a very good idea of the stability of the factor solution can be obtained.

Aside from the important role of correlations in data reduction, they have a place in the testing of hypotheses, particularly at the exploratory stage. Thus we will examine the intercorrelations of oxygen delivery system components, serum thiocyanate and cotinine levels, and erythropoietin levels (specific goals A.4 and A.9) and, similarly, cardiovascular, hematologic, pulmonary and hemorheologic alterations with the duration and extent of passive smoking exposure (specific goal A.9). An interesting twist to the correlation analyses will be provided by using canonical correlation analysis to identify the linear composite of the outcome variables which has the highest correlation with all the indices of passive smoking taken jointly. This will identify a canonical variate which may best illustrate the other analyses of the impact of passive smoking. These analyses will be implemented using the CANCORR procedure in SAS.

f. Genetic influences and the effects of passive smoking: regression analysis

The ANOVA outlined in Section 2.D.6.c recognizes the important distinction between variation which arises within families and variation which differentiates one family from another. It does not address the causes of this variation and does not exploit the fundamental difference between MZ and DZ twins to distinguish genetic and environmental influences.

One reason why the resolution of between and within family variation may be useful in gaining a proper perspective in epidemiological studies is that conclusions about the importance of risk factors may depend on the relative importance of genetic effects. A plausible partitioning of the variation in an oxygen delivery system variable may show 40% arising between families and 60% within families. Assuming additive genetic and environmental contributions, the variation may further break down into 30% and 10% for the genetic and environmental components between families, and 30% and 30% for the genetic and environmental components within families. If we take passive smoking effects on children to be a between families environmental effect, an overall influence of 5% of variance associated with passive smoking is now seen to account for fully 50% of the environmentally determined variation between families. It may well turn out that most of the variation which can be influenced by parents changing their behavior (e.g. smoking or diet) is accounted for by a few factors which do not, on a superficial epidemiological analysis, account for a large proportion of the overall variation.

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In Section 2.D. g and Section 3 Append III we shall present structural equation model approach to the analyses. Here is described a powerful and elegant preliminary analysis to test for genetic and environmental variation and for the impact of the defined environmental index (passive smoking) on the mean expression of the dependent variables, on the genetic variation in the dependent variables and on the influences of familial environments other than those associated with the index itself. Thus as well as the main effects, this provides a test for an important type of genotype X environmental interaction; Are some genotypes particularly susceptible to the effects of passive smoking, or does passive smoking either enhance or suppress the expression of normal genetic variation in the determinants of systemic oxygen delivery and possibly increase the risk for disease production?

The general analytic strategy (60-62) can be illustrated by considering a single dependent variable, diastolic blood pressure (DBP) for example. To test for familial aggregation Twin A's DBP is regressed on that of Twin B. A significant regression is evidence for familial aggregation. To separate genetic and environmental influences a dummy zygosity variable (Z) is created taking the value 1.0 if the twins are MZ and 0.5 if they are DZ. The partial regression coefficients from the multiple regression of Twin A's score on Twin B's score, the zygosity variable (Z) and the product (Z X B) of Twin B's score and Z provide estimates of and tests for familial environment, mean difference between MZ and DZ twins (expected to be zero), and genetic effects respectively.

The power of our study design now becomes apparent since the familial environment is not a "black box" of unknown influences. There is an index (smoking in the parents) of a putative component of the general familial environment affecting the dependent variables. We can therefore extend the analysis to include this as an independent variable (together with its interactions with B and Z). The full analysis is outlined in Table 6.3 where we have indicated what we would expect the outcome of the analysis to be on the simple hypothesis that there are genetic and environmental contributions to familial aggregation which act additively, but that all of these environmental influences are associated directly with passive smoking.

This form of analysis may be extended to include categorical variables like sex and race or quantitative covariates which may be family history variables or scores on some other variable. However, given a sample size of 300 families we will be cautious in the inclusion of multiple predictors. The analysis is readily implemented using SSPS regression procedures or SAS GLM. It will provide specific tests of hypothesis A.1 and specific goal A.10 as well as a range of subhypotheses.

g. Genetic, environmental, multivariate and developmental models:  
Maximum likelihood modelling covariance structures

Although traditional regression approaches have an immediate appeal and as shown in Section 2.D.6.f can be adapted to test particular hypotheses in this study, an alternative procedure is more appropriate for more complex genetic and environmental parameter estimation, particularly when we want to allow for specified structural relationships between latent variables or predictor variables. The maximum likelihood linear structural equation modelling exemplified in LISREL VI (63) or in analyses developed specifically for genetically informative data sets (64) is a flexible but rigorous analytic strategy for estimating the parameters of structural models (path models) and for deciding between alternative models. It can be adapted to the analysis of twin and family data on multiple variables and to the analysis of longitudinal developmental data. (65) An introduction to some general features of this approach is given in

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Section 3 Appendix .1 where we illustrate genetic and environmental models for body weight, left ventricular mass and their interrelationship.

This analytic method can be readily extended to other tests of multivariate structural hypotheses, tests of sex differences in gene expression or developmental paths, models of parent offspring resemblance and tests of the impact of passive and active smoking on outcome variables. In the latter case the correlations between smoking, scored as a dichotomy, and other variables may be entered as a point biserial correlation. Alternatives to this, e.g. estimates of the biserial correlation, would require additional and probably implausible assumptions about the effects of a continuously distributed latent variable which influences the smoking habit. However, where appropriate, tetrachoric or polychoric correlations may be estimated by the LISREL VI package for subsequent structural modelling.

Table 6.3 Multiple regression analysis for twin data with an environmental index.

Regression model:

$$A = c_{\text{nst}} + b_{1B} + b_{2Z} + b_{1BZ} + b_{4S} + b_{5NS} + b_{6ZS} + b_{7BZS}$$

where A= Twin A's score  
B= Twin B's score  
Z= Zygosity coded 1 or -1  
S= Smoking coded 1 or -1

PREDICTOR	INTERPRETATION OF SIGNIFICANT PARTIAL REGRESSION	EXPECTED OUTCOME
Passive smoking (S)	Passive smoking influences outcome	$b_4 < 0$
Zygosity (Z) and ( $2 \times S$ )	Differences between average score of MZ twins and DZ twins	$b_2, b_6 < 0$
Twin B (B)	Familial environment influences outcome, over and above effects of passive smoking	$b_1 = 0$
( $B \times Z$ )	Genetic effects on outcome	$b_3 > 0$
( $B \times S$ )	Passive smoking alters impact of B	$b_5 = 0$
( $B \times Z \times S$ )	Passive smoking alters expression of genetic variation. Are genotypes differentially susceptible to passive smoking?	$b_7 = 0$

\* Expected if all familial environmental effects are due to passive smoking and these act additively with genetic variation.

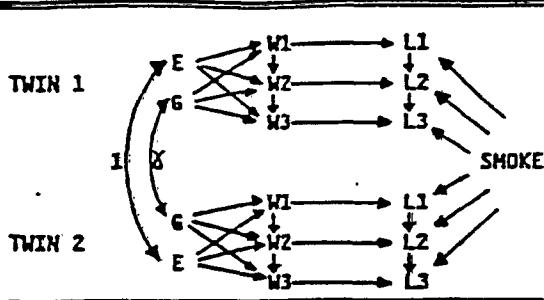
A skeleton of a developmental model linking passive smoking (S) to body weight (W) and left ventricular mass (L) is shown in Figure 6.1. In this figure the residual environmental paths to W and to L have been omitted. This simplified model assumes that one common set of genetic effects acts on W at all three occasions of measurement (ages 11, 12.5 and 14 years), together with one common set of environmental influences shared by members of a twin pair. There are no age specific genetic influences on W and no genetic influences on L except those mediated by W. However L is influenced by passive smoking status (S), which is shared by members of a twin pair. There are also developmental influences of earlier measures on later measures. (65,66) Incremental effects of passive smoking will be detected by the relative decline in magnitude of the path from W to L over the three ages. A sample program for fitting this model, together with illustrative output for a simulated variance-covariance matrix is given in Section 3 Appendix III.

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Figure 6.1 A developmental model incorporating the effects of passive smoking.



( $\gamma = 1.0$  for MZ pairs and 0.5 for DZ pairs;  $W_1, W_2, W_3$  are body weights at ages 11, 12.5 and 14 years;  $L_1, L_2, L_3$  are left ventricular masses at ages 11, 12.5 and 14 years;  $G$ = genetic effects on weight;  $E$ =shared family environmental effects on weight;  $SMOKE$ = shared passive smoking effects on left ventricular mass.)

The replicate observations provided by members of a twin pair are helpful in assessing the ability of a regression model to predict criterion variables such as LV mass or DBP from independent variables such as smoking status (fixed effects) and covariates such as body weight (random effects). The adequacy of the predictor variables (independent variables and covariates) taken together is indexed by the squared multiple correlation ( $R^2$ ) between the predictor variables and the criterion; the independent contribution of a particular variable may be judged by how much it increments  $R^2$ . This analysis focuses on the interclass correlations as a function of the independent variables of interest and may be implemented using, for example, a stepwise multiple regression procedure (e.g. PROC STEPWISE in SAS) which provides the relevant statistics and appropriate tests of significance.

However, the success of the prediction equation may be overestimated in any particular sample because we 'capitalize on chance' associations in the sample data. The prediction equation uses regression weights which maximize the correlation between predicted and observed values. Thus to avoid an overoptimistic interpretation of the success of the prediction equation, a recommended safeguard is to test the prediction equation on another sample not used in deriving the regression weights. Short of recruiting a new sample, and doubling the size and cost of the study, we may randomly select one member from each twin pair to serve as our 'estimation' sample to derive the prediction equation and use the other member of each pair as our 'test' sample to evaluate the  $R$ . Although these two samples are not independent, in as much as the  $R^2$  was inflated in the 'estimation' sample purely by the association of random individual errors of measurement, so the  $R$  will be corrected downwards in the 'test' sample. This makes use of the replication aspect of twins to provide a check on over interpretation of the data.

As an illustration of this we looked at the prediction of resting systolic blood pressure (SBP) from age, resting heart rate and weight in one twin from each of 231 complete pairs in our pilot sample. The  $R^2$  was 0.23 and was highly significant ( $p < .0001$ ). Using the derived prediction equation to predict the SBPs of the second twin from each pair resulted in an  $R$  of 0.21 which was, of course, similarly significant. We concluded that these predictor variables account for over 20% of the variation in SBP and that this level of prediction is not a consequence of associated random errors of measurement in the sample used to derive the prediction equation.

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This structural modelling approach to the ta will be used to test all the hypotheses in Section 2.A and specific goals A.4, A.6, A.9 and A.10. It is a general method suitable for integrating genetic, environmental, multivariate and developmental models.

**h. Summary of Analysis**

We have sketched the initial approaches to the data analysis. Experience with the collected data may suggest new approaches to the analyses. The directions taken in data analysis are to a considerable extent dependent on the current hypotheses and theoretical positions adopted. We have demonstrated how each of the general hypotheses and those implied by the specific goals will be tested, but it is expected that the analytic strategies will evolve during the project as a result of informed criticism and in response to new theoretical developments.

**7. Experimental Problems**

No significant experimental problems exist nor are any expected to arise which would need to be overcome in order to successfully accomplish the goals of this research project.

**E. Human subjects**

**1. Subject Population**

The subjects will consist of healthy monozygotic and dizygotic twins, who will be 11-years old at the beginning of the study, and their parents. The twins will be ascertained from a population-based registry.

**2. Research Material**

The sources of research material will include data obtained from anthropometric evaluation, pulmonary function tests, exercise testing, echocardiography and from a health questionnaire completed by the parents. Samples of venous blood will be obtained from each subject. The data and laboratory results will be used specifically for research purposes.

**3. Subject Recruitment**

The families of twins meeting the age and geographic criteria (Central Virginia) for inclusion are contacted initially by the Middle school the twins attend to maintain confidentiality from the investigators. The parents reply by mail and after a telephone followup from a research associate in the Division of Pediatric Cardiology, are invited for participation. After a full oral description of the study's longitudinal nature and the tests to be performed, written consent is obtained.

**4a. Risk to children**

The only risk of drawing blood in children is a hematoma (black and blue bruise). The risk of exercise in children is negligible but they will be monitored by blood pressure and electrocardiogram. Technicians will be taught to recognize abnormal heart rhythms.

**4b. Risk to parents**

Before exercise, adults will be questioned about chest pain, shortness of breath or a history of cardiovascular disease. No subjects with any of these symptoms will undergo dynamic exercise. Adults who are on treatment for hypertension will undergo dynamic exercise if their systolic blood pressure is less than 160mm Hg and their diastolic blood pressure less than 105mm Hg. All of the exercise tests on adults will have blood pressure and electrocardiographic monitoring by a physician.

**5. Minimizing Risks**

Strict adherence to the above guidelines for exercise-testing adults will minimize the low risk of exercise associated difficulties. In the event of such difficulties, the exercise laboratory contains emergency equipment and personnel experienced in its use. Each individual is assigned a family and individual number and all of the acquired data is encoded by these numbers to maintain confidentiality.

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**6. Benefits**

The benefits of the study are both specific and general. Individual families will benefit from an accurate determination of the zygosity of their twins and the presymptomatic abnormalities detectable by echocardiography, spirometry or electrocardiography. Abnormalities detected in the twins as well as their parents which are related to cigarette use may provide the impetus for cessation of cigarette smoking. General benefits from this study include improved methods for detecting the childhood antecedents of adult cardiovascular and pulmonary diseases. We believe that the general and specific benefits to be derived from the study far outweigh any potential hazard.

**F. Vertebrate Animals**

No laboratory animals will be used in this research.

**G. Consultants**

Dr. Richard M. Schieken, Professor and Chairman of the Division of Cardiology, Department of Pediatrics of the Medical College of Virginia will serve as the Cardiology Consultant. Dr. Walter E. Nance, Professor and Chairman of the Department of Human Genetics of the Medical College of Virginia will serve as a Genetics Consultant. Dr. Lindon Eaves also of the Department of Human Genetics will serve as a Genetics Consultant, with emphasis in the genetic epidemiology aspects of the project. Dr. Michael Mosteller has his degree in genetics and is the Data Manager for the ongoing Longitudinal Twin Study. He will serve as the Data Manager Consultant for the project and aid the co-investigators in organizing data acquisition, maintaining the data sets and working with the co-investigators to provide material for analysis. Letters of agreement are included.

**H. Consortium Arrangements**

None.

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## Department of Pediatrics Children's Medical Center

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September 11, 1986

William B. Moskowitz, M.D.  
Assistant Professor of Pediatrics  
Division of Pediatric Cardiology  
Box 543  
Medical College of Virginia  
Richmond, Virginia 23298-0001

Dear Dr. Moskowitz:

It is with great pleasure that I agree to serve as a consultant in Cardiovascular Epidemiology for you in your grant "Childhood Passive Smoking: Cohort Study of Cardiac Risk."

Sincerely,

Richard M. Schieken, M.D.  
Professor and Chairman  
Division of Pediatric Cardiology

RMS/clh

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Medical College of Virginia  
Virginia Commonwealth University

September 4, 1986

Dr. William Moskowitz  
Assistant Professor  
Department of Pediatrics  
P.O. Box 26, MCV Station  
Richmond, Virginia 23298

Dear Bill:

This letter is to document my willingness to serve as a consultant on the grant you are preparing to investigate the possible effects of active and passive smoking in the families of adolescent twins. The preliminary data you have obtained suggesting that there may be an effect of passive smoking is exciting and the research design you plan to employ involving twins and their families probably offers the best hope of disentangling the complex interactions of genetic and environmental effects on the outcome variables you will be examining.

Yours sincerely,

*Walter E Nance, M.D.*  
Walter E. Nance, M.D., Ph.D.  
Chairman and Professor  
Department of Human Genetics

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ARNOLD SALOMON, M.D.  
THOMAS M. BRUNNEMEIER, M.D.

URONLOGY  
BRUCE BROCKER, M.D.

William B. Moskowitz, MD  
Assistant Professor  
Pediatric Cardiology  
Box 543, MCV Station

Richmond, VA 23298-0543

Dear Bill:

I would be pleased to serve as data management consultant for your project, "Childhood passive smoking: A Cohort Study of Cardiac Risk".

I have enjoyed working with you over the past year and a half on preliminary investigations of the effects of passive smoking on adolescent twins. I now look forward to assisting you as you build on your previous work and test hypotheses which I believe are both relevant to public welfare and scientifically interesting.

Sincerely,

*Michael Mosteller, Jr.*

Michael Mosteller, Jr., Ph.D.  
Assistant Professor  
Department of Family Practice  
Box 251, MCV Station

MM/jlp

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Medical College of Virginia  
Virginia Commonwealth University

Dr W. Moskowitz,  
Division of Pediatric Cardiology,  
Medical College of Virginia.

September 16th 1987.

Dear Bill,

I am delighted to act as advisor and collaborator in your proposed study of passive smoking in adolescent twins. It is a neat idea to exploit the ongoing twin-parent study for this purpose because the twin design allows us to say so much more about why individuals differ in their response to environmental insults. With John Hewitt's help, we can address these issues in the design and analysis of the investigation.

I have enjoyed the collaboration between our Genetic Epidemiology group and yourselves over the last five years. I believe it has been a model of what can be achieved when complementary strengths are combined in a study. Our regular meetings about the longitudinal twin study have been fruitful and stimulating. I am glad to have a further incentive to continue.

--  
I hope this new venture will build on what we have already achieved together and wish you every success with your proposal.

Best wishes,

A handwritten signature in black ink, appearing to read "LJ Eaves".

Lindon J Eaves, MA(Oxon), PhD, DSc.  
Distinguished Professor,  
Department of Human Genetics.

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